Bima Journal of Science and Technology, Vol. 7 (2) June, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i2.427

STRUCTURE AND CATALYTIC RESIDUE PREDICTION OF A DEHALOGENASE FROM THE THIRD DOMAIN OF LIFE

[1]*HASSANA ABUBAKAR, [2]RABIU SALIHU, [1]YAKUBU NDATSU, [1]AMUZAT ALIYU OLALEKAN, [1]HAMZA USMAN BOKO, [1]HADIZA MOHAMMED and[1]MARY LADIDI ABU

[1]Department of Biochemistry, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University Lapai, PMB 11, Lapai, Niger State, Nigeria
[2]Department of Biological Sciences, Federal University Dutse, PMB 7156 Dutse Jigawa State, Nigeria

*Corresponding Author: ahassana@ibbu.edu.ng

ABSTRACT

Extreme archaea are known for their distinct molecular identities and production of enzymes with unique activities that have accorded them diverse applications. The amino acid sequence of most of these enzymes are deposited in the databank with unpredicted structures and domains. Here, the secondary structure, domain and three-dimensional structure of a dehalogenase from a halophilic archae was predicted employing GOR4, Pfam and four different modeling servers respectively. Model validation was performed in SAVES 06 based on Ramanchandran plot and ERRAT analysis. PyMOL sofware was used to visualize the model and superimpose it into the template. 45.93 % alpha helix, 15.85% extended strand and 38.21% random coil were the secondary structures predicted while HAD 2 was the only domain found in this enzyme. The SWISS-MODEL result was found to be the best model with the highest percentage of 92.9% residues in the most favoured region based on the Ramanchandran plot, and an overall quality factor of 97.196% in the ERRAT analysis. Aspartic acid was identified as essential catalytic residue. This structure prediction will help in the engineering of the enzyme for bioremediation application.

Keywords: Halophilic Archaea, Dehalogenase, Structure, Catalytic Residue, Third Domain

INTRODUCTION

Halophilic archaea (haloarchaea) are members of the Archaea, the third domain of life. These organisms are salt-loving and have developed mechanisms to survive in hypersaline environments of 3-5 M NaCl concentrations (Oren, 2014; Yang et al., 2007). Generally, these microorganisms grow between 15 °C to approximately 45–50 °C with optimum growth at 37 °C, but most of their enzymes perform well above this range (Garrity and Stanley 2001; Litchfield, 2011).

Extreme Archaea are reported to possess unique metabolic pathways which can produce enzymes with unique activities and applications (Siti, Nurul, et al., 2018; Kumar et al., 2011). Earlier reviews outlined various enzymes of haloarchaea discovered for different biotechnological applications such as hydrocarbon degradation, biofuel production, saline wastewaters remediation, among others(Kasirajan and Maupin-Furlow, 2021; Litchfield, 2011). In recent studies, a dehalogenase has also been discovered as one of the unique enzymes produced by these organisms (Podell et al., 2013).

Dehalogenases are enzymes that catalyze the essential step in the degradation of halogenated organic compounds by cleaving the carbon-halogen bonds to release the halide ions (Hamid *et al*., 2011; Janssen *et al*., 2001). These enzymes are typically grouped as

DOI: 10.56892/bima.v7i2.427

hydrolytic, reductive and oxygenolytic dehalogenases (Fetzner and Lingens, 1994; Oyewusi *et al*., 2020a).

However, Hill et al. (1999) later classified them based on their gene families into group I and II.They have been extracted from various soil microorganisms including *Burkholderia pseudomallei* MF2, *Delftia acidovorans*, *Bacillus subtilis* strain H1, *Bacillus thuringiensis* strain H2, *Pseudomonas stutzeri* DEH130 and etcetera (Edbeib *et al*., 2020; Harris *et al*., 2022; Oyewusi *et al*., 2020b; Zhang *et al*., 2013). Their isolation was not only limited to soil but also from marine and extreme environments (Alomar et al., 2014; Novak et al., 2013; Sitiet al., 2018).

Microorganisms use halogenated compounds as carbon and energy source or/and as a way of protecting themselves against the toxicity of these compounds (Alomar et al., 2014; Müller and Lingens, 1986). Hence, they aid in the removal of these compounds from the environment. The significance of dehalogenases in the bioremediation of toxic halogenated compounds prompted researchers into documenting their three- dimensional structures to have a better knowledge of their folds, interactions and functions.

Most of the structures reported so far are from bacteria domain of life. Thus, the three-dimensional structure of a dehalogenase from an Archaeon became the focus of this study. This dehalogenase structure is the first prediction from this domain of life to the best of our knowledge and this will help in the manipulation of this enzyme for bioremediation technology.

MATERIALS AND METHODS

Sequence Retrieval, Secondary Structure And Domain Prediction

The amino acid sequence of Halophilic Archaeon (J07HX64) dehalogenase was obtained from Uniprot (Boutet et al., 2016) with accession number (U1PEQ1). The sequence was submitted to NPS@: GOR4 (Combet et al., 2000) for secondary structure prediction. The sequence was further submitted to Pfam server (Bateman et al., 2004) for domain prediction.

3D Structure Prediction and Validation

The three-dimensional structure of the Dehalogenase was modeled employing I-TASSER (Zhang, 2008) server, SWISS-MODEL (Bordoli et al., 2006), Raptor x (Källberg et al., 2012), and PHYRE2 (Kelley et al., 2014). The pdb files generated from the modeling servers were submitted to PROCHECK for Ramachandran plot. Ramachandran plot was carried out to check the stereochemical features of the models The best model from Ramachandran plot analysis was further analyzed using ERRAT.

Model Visualization and Active Site Prediction

The model superimposition with the template (PDB ID:2no4) and active site identification was carried out with PyMOL software.

RESULTS AND DISCUSSION

Amino Acid Sequence, Secondary Structure and Domain

The dehalogenase from Halophilic Archaeon J07HX64 consists of 246 amino acid residues. The secondary structure of these residues as predicted by NPS@: GOR4 is shown in figure 1. Alpha helix (Hh) is 113 residues (blue), Extended strand (Ee) is 39 residues (red) and Random coil (Cc) is 94 residues (orange) having 45.93 %, 15.85% and 38.21% respectively. This result is in line with the

DOI: 10.56892/bima.v7i2.427

preceding dehalogenase secondary structure predictions. Dehalogenases have been reported to be composed mainly of alpha helices, some coil and few or no beta strands (Hamid et al., 2013; Sudi et al., 2012).

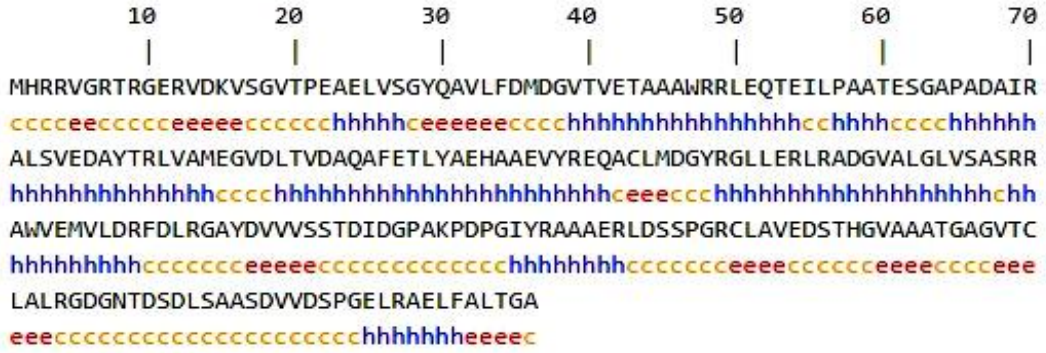

Figure 1: Secondary structure prediction result. Blue color is Alpha helix (Hh), red color is Extended strand (Ee) and orange color is Random coil (Cc).

The only domain found in the dehalogenase from halophilic archaeon is Haloacid dehalogenase like hydrolase (HAD 2) in green color from residue 32 to 213 (figure 2). The arrow pointers (pink) are the predicted active sites at position 35 and 37 for first and second pointer respectively. HAD 2 domain predicted is evidence that this enzyme truly belongs to HAD family. DehIVa (*Burkholderia cepacia* MBA4), DehI (*Pseudomonas putida* PP3) and DehE (*Rhizobium sp.* RC1) have also been reported to belong to HAD family (Schmidberger et al., 2007; Schmidberger et al., 2008; Hamid et al., 2013). HAD family is a collection of enzymes including phosphatases, phosphon atases, P-type ATPases, beta-phosphoglucomutases, phosphomannomutases , and dehalogenases which participate in various cellular processes, from amino acid biosynthesis to detoxification (Koonin and Tatusov, 1994; Srinivasan, 2011).

Figure 2: HAD 2 domain (green) in dehalogenase from halophilic archaeon J07HX64 predicted by Pfam server. The arrow pointers (pink) are the predicted active sites.

3D Structure Prediction and Validation

The models from I-TASSER, SWISS-MODEL, RaptorX, and PHYRE2 have 72.4%, 92.9%, 92.1% and 85.0% residues in most favored regions respectively. A quality model is expected to attain 90% and above in most favored region as explained in figure 3. Though SWISS-MODEL and RaptorX Ramachandran plot results are > 90%, the earlier has higher residues in the most favored regions, hence emerge best in this study. It has 92.9%, 6.6%, 0.5% and 0.0% residues in the most favored, additional allowed, generously allowed and disallowed regions respectively (figure 3).

DOI: 10.56892/bima.v7i2.427

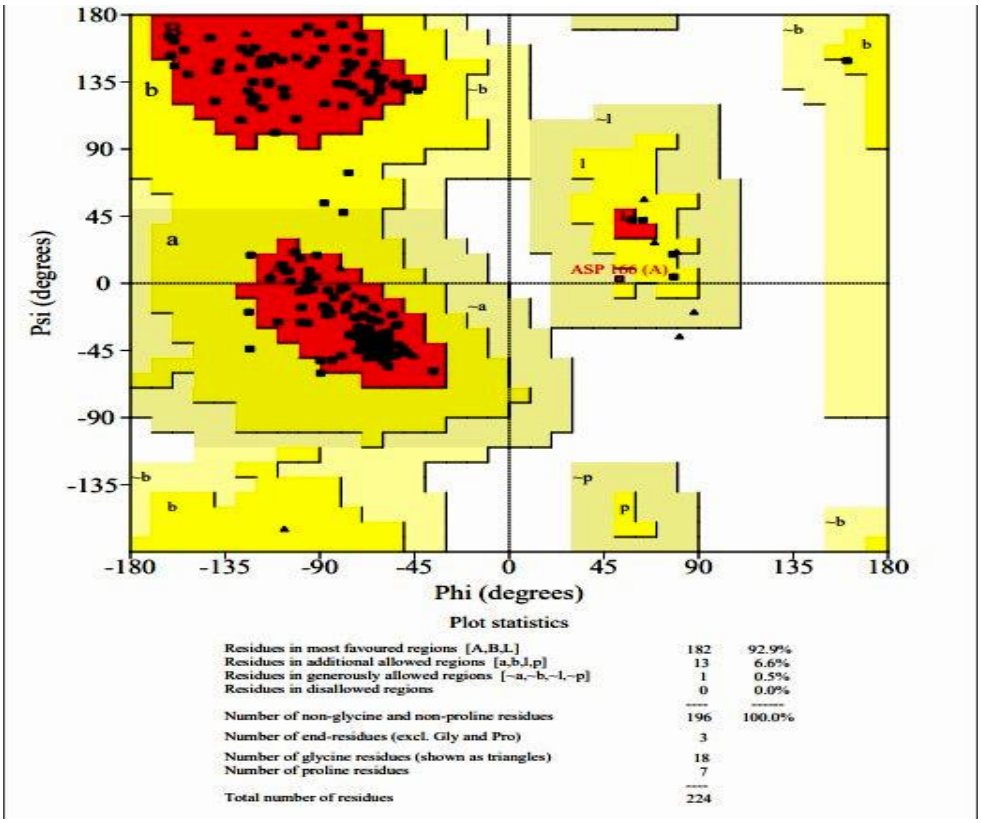

Figure 3: Ramanchandran plot of 3D structure predicted by swizz model.

Further validation with Errat produced 97.196% overall quality factor (figure 4). Good quality resolution structures are said to generate 95% or higher values in general. Models of a dehalogenase and Ribokinase from SWISS-MODEL have been reported among others to be of good quality (Hamid et al., 2013; Abubakar et al., 2021; Oyewusi et al., 2022).

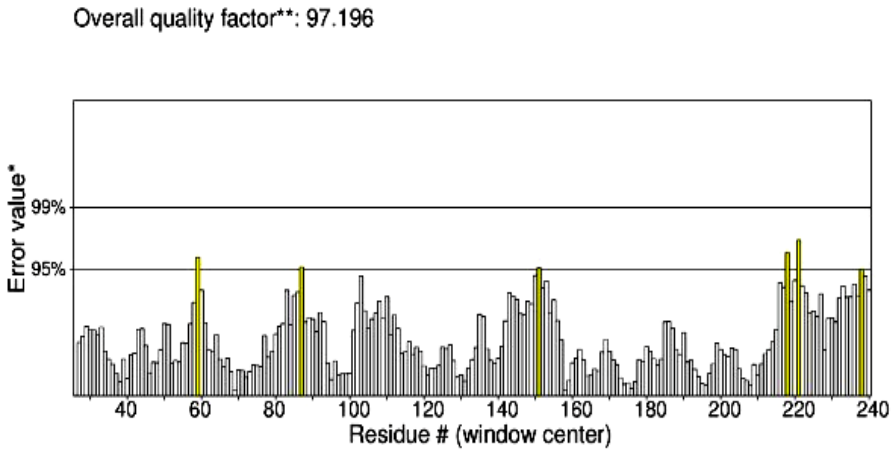

Figure 4: Errat analysis result showing good quality factor.

Bima Journal of Science and Technology, Vol. 7 (2) June, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i2.427

Model Visualizationand Active Site

Just as predicted by GOR4 server in secondary structure prediction that this enzyme has more alpha helices, some random coil and little extended strands in figure 1 above, it is apparent in the tertiary structure when viewed with PyMOL (figure 5).

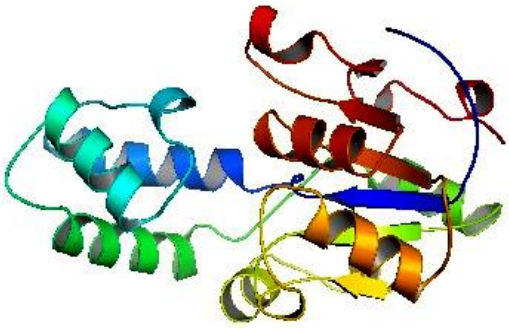

Figure 5: Cartoon view of 3 D structure of a dehalogenase from halophilic archaeon J07HX64 predicted by SWISS-MODEL server.

The active site residues identified are shown in figure 6a. Previously, Aspartate (Asp) has been described as a vital nucleophile in the dehalogenation process (Adamu et al., 2020; Hamid et al., 2013). Fortunately, when our model was superimposed into the template (DehIVa), the aspartic acid residue at position 35 of our model aligned with Asp11 (figure 6b) which was discovered in DehIVa as an important nucleophile(Schmidberger et al., 2007). Asp194, Asp189, Asp189 and Asp125 were identified in DehI, DL-DEX113, DehE and DehH2 respectively (Nardi-Dei et al., 1997; Schmidberger et al., 2008; Hamid et al., 2013; Abidin *et al*., 2019; Oyewusi et al., 2022). This signifies that specific aspartic acid residues act as the catalytic residue in this class of enzymes but are found at different positions in different dehalogenases. In this study, it was found at position 35 which was one of the positions predicted as the active site by Pfam tool.

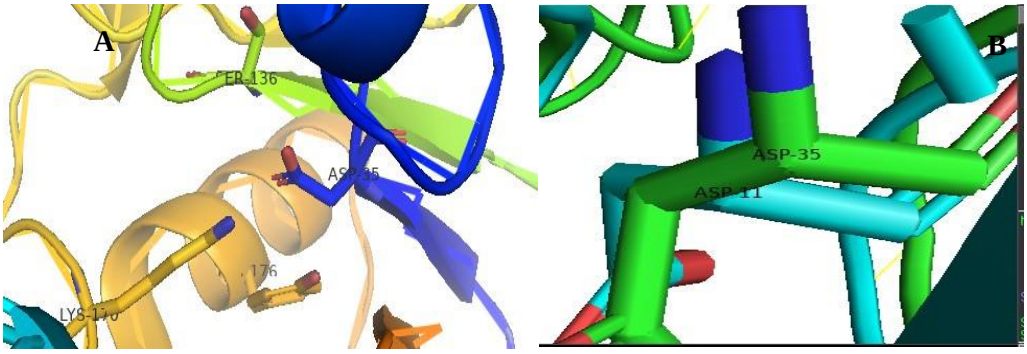

Figure 6: (a) The active site residues are shown as Sticks (b) superimposition of the catalytic residue; Model (green) and template (blue).

CONCLUSION

This is a good quality model and the first dehalogenase structure from the third domain of life. This enzyme showed similar domains, secondary structure folds with bacterial dehalogenases and also engaged the same essential residue for catalysis. This study will help in the manipulation of this enzyme for bioremediation technology in order to attain cleaner environment.

REFERENCES

Abidin, M. H. Z., Abd Halim, K. B., Huyop, F., Hamid, T. H. T. A., Wahab, R. A., and Hamid, A. A. A. (2019). The mechanistic role of active site residues in non-stereo haloacid dehalogenase E

DOI: 10.56892/bima.v7i2.427

(DehE). *Journal of Molecular Graphics and Modelling*, *90*, 219–225.

Abubakar, H., Ndatsu, Y., Musa, A. D., Ogbiko, C., Usman, H. B., Mayaki, F. G., and Mohammed, A. (2021). In silico Structural Modelling of Ribokinase from Salmonella Typhi. *Earthline Journal of Chemical Sciences*, *5*(1), 191-206.

Adamu, A., Wahab, R. A., Aliyu, F., Aminu, A. H., Hamza, M. M., and Huyop, F. (2020). Haloacid dehalogenases of Rhizobium sp. and related enzymes: Catalytic properties and mechanistic analysis. *Process Biochemistry*, 92, 437–446.

Alomar, D., Abdul Hamid, A. A., Khosrowabadi, E., Gicana, R. G., Lamis, R. J., Huyop, F. and Tengku Abdul Hamid, T. H. (2014). Molecular Characterization of Monochloroacetate-Degrading Arthrobacter sp. Strain D2 Isolated from Universiti Teknologi Malaysia Agricultural Area. *Bioremediation Journal*. 18(1), 12-19.

Bateman, A., Coin, L., Durbin, R., Finn, R. D., Hollich, V., Griffiths-Jones, S., and Eddy, S. R. (2004). The Pfam protein families database. *Nucleic acids research*, 32(suppl_1), D138-D141.

Bordoli, A. L., Kopp, J., and Schwede, T. (2006). The Swiss-MODEL Workspace: a web-based environment for protein structure homology modeling. *Bioinformatics*, 22, 195-201.

Boutet, E., Lieberherr, D., Tognolli, M., Schneider, M., Bansal, P., Bridge, A. J., and Xenarios, I. (2016). UniProtKB/Swiss-Prot, the manually annotated section of the UniProt KnowledgeBase: how to use the entry view. In Plant Bioinformatics (pp. 23-54). Humana Press, New York, NY.

Combet, C., Blanchet, C., Geourjon, C., and Deleage, G. (2000). NPS@: network protein sequence analysis. *Trends in biochemical sciences*, 25(3), 147-150.

Edbeib, M. F., Wahab, R. A., Huyop, F. Z., Aksoy, H. M., and Kaya, Y. (2020). Further analysis of Burkholderia pseudomallei MF2 and identification of putative dehalogenase gene by PCR. *Indonesian Journal of Chemistry*, *20*(2), 386–394.

Fetzner, S., and Lingens, F. (1994). Bacterial dehalogenases: biochemistry, genetics, and biotechnological applications. *Microbiological Reviews*. 58(4), 641.

Garrity, G. M., and Stanley, J. T., 2001. Bergey's Manual® of Systematic Bacteriology, in: Boone, D. R., Castenholz R. W. (Eds.), The Archaea and the Deeply Branching and Phototrophic Bacteria. Springer, New York.

Hamid, A. A. A., Wong, E. L., Joyce-Tan, K. H., Shamsir, M. S., Hamid, T. H. T. A., and Huyop, F. (2013). Molecular modelling and functional studies of the non-stereospecific α-haloalkanoic acid dehalogenase (DehE) from Rhizobium sp. RC1 and its association with 3-chloropropionic acid (β-chlorinated aliphatic acid). *Biotechnology and Biotechnological Equipment*, 27(2), 3725-3736.

Hamid, T. H. T. A., Hamid, A. A. A., and Huyop, F. (2011). A review on non-stereospecific haloalkanoic acid dehalogenases. *African Journal of Biotechnology*, *10*(48), 9725–9736.

Harris, J. D., Coon, C. M., Doherty, M. E., McHugh, E. A., Warner, M. C., Walters, C. L., Steel, J. J., 2022. Engineering and characterization of dehalogenase enzymes from Delftia acidovorans in bioremediation of perfluorinated compounds. *Synth. Syst.*

DOI: 10.56892/bima.v7i2.427

Biotechnol. 7, 671-676.

Hill, K. E., Marchesi, J. R., and Weightman, A. J. (1999). Investigation of two evolutionarily unrelated halocarboxylic acid dehalogenase gene families. *Journal of Bacteriology*, *181*(8), 2535–2547.

Janssen, D. B., Oppentocht, J. E., and Poelarends, G. J. (2001). Microbial dehalogenation. *Current Opinion in Biotechnology*. 12(3), 254-258.

Källberg, M., Wang, H., Wang, S., Peng, J., Wang, Z., Lu, H., and Xu, J. (2012). Template-based protein structure modeling using the RaptorX web server. *Nature protocols*, 7(8), 1511-1522.

Kasirajan, L., and Maupin-Furlow, J. A. (2021). Halophilic archaea and their potential to generate renewable fuels and chemicals. *Biotechnology and Bioengineering*, *118*(3), 1066–1090.

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., and Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature protocols*, 10(6), 845-858.

Koonin, E. V., and Tatusov, R. L. (1994). Computer analysis of bacterial haloacid dehalogenases defines a large superfamily of hydrolases with diverse specificity: application of an iterative approach to database search. *Journal of molecular biology*, 244(1), 125-132.

Kumar, L., Awasthi, G., and Singh, B. (2011). Extremophiles: a novel source of industrially important enzymes. *Biotechnology*, 10(2), 121-135. https://scialert.net/abstract/?doi=biotech.2011.121.135.

Litchfield, C. D. (2011). Potential for industrial products from the halophilic Archaea. *Journal of Industrial Microbiology and Biotechnology*, 38(10), 1635.

Müller, R., and Lingens, F. (1986). Microbial Degradation of Halogenated Hydrocarbons: A Biological Solution to Pollution Problems? *Angewandte Chemie International Edition in English*. 25(9), 779-789.

Nardi-Dei, V., Kurihara, T., Park, C., Esaki, N., and Soda, K. (1997). Bacterial DL-2-haloacid dehalogenase from Pseudomonas sp. strain 113: gene cloning and structural comparison with D-and L-2-haloacid dehalogenases. *Journal of bacteriology*, 179(13), 4232-4238.

Novak, H. R., Sayer, C., Panning, J., and Littlechild, J. A. (2013). Characterisation of an l-haloacid dehalogenase from the marine psychrophile Psychromonas ingrahamii with potential industrial application. *Marine biotechnology*. 15(6), 695-705.

Oren, A. (2014). Halophilic archaea on Earth and in space: growth and survival under extreme conditions. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *372*(2030), 20140194.

Oyewusi, H. A., Huyop, F., Wahab, R. A., 2022. Molecular docking and molecular dynamics simulation of Bacillus thuringiensis dehalogenase against haloacids, haloacetates and chlorpyrifos. *Journal of Biomolecular Structure Dynamic* 40, 1979-1994.

Oyewusi, H. A., Wahab, R. A., Huyop, F., 2020a. Dehalogenase-producing halophiles and their potential role in bioremediation. Mar. Pollut. Bull. 160, 111603.

Oyewusi, H. A., Wahab, R. A., Kaya, Y., Edbeib, M. F., and Huyop, F. (2020b). Alternative bioremediation agents

DOI: 10.56892/bima.v7i2.427

against haloacids, haloacetates and chlorpyrifos using novel halogen-degrading bacterial isolates from the hypersaline lake Tuz. *Catalysts*, *10*(6), 651.

Podell, S., Ugalde, J. A., Narasingarao, P., Banfield, J. F., Heidelberg, K. B., and Allen, E. E. (2013). Assembly-driven community genomics of a hypersaline microbial ecosystem. *PloS one*, 8(4), e61692.

Schmidberger, J. W., Wilce, J. A., Tsang, J. S., and Wilce, M. C. (2007). Crystal structures of the substrate free-enzyme, and reaction intermediate of the HAD superfamily member, haloacid dehalogenase DehIVa from Burkholderia cepacia MBA4. *Journal of molecular biology*, 368(3), 706-717.

Schmidberger, J. W., Wilce, J. A., Weightman, A. J., Whisstock, J. C., and Wilce, M. C. (2008). The crystal structure of DehI reveals a new α-haloacid dehalogenase fold and active-site mechanism. *Journal of molecular biology*, 378(1), 284-294.

Siti, N., Arman, S., Mohamed, F. E., Aliyu, A., Firdausi, A., Roswanira, A. W., and Fahrul, H. (2018). Dehalogenase producing bacteria from extreme environment: a review. *Malaysian journal of microbiology*, 14(5), 424-434.

Srinivasan, B. (2011). Structure-function studies on three members of the haloacid dehalogenase (HAD) superfamily of enzymes (Doctoral dissertation, Jawaharlal Nehru Centre for Advanced Scientific Research).

Sudi, I. Y., Wong, E. L., Joyce-Tan, K. H., Shamsir, M. S., Jamaluddin, H., and Huyop, F. (2012). Structure prediction, molecular dynamics simulation and docking studies of D-specific dehalogenase from Rhizobium sp. RC1. *International Journal of Molecular Sciences*, 13(12), 15724-15754.

Yang, Y., Levick, D. T., and Just, C. K. (2007). Halophilic, thermophilic, and psychrophilic archaea: Cellular and molecular adaptations and potential applications. *J. Young Invest.* 17(4). https://www.jyi.org/2007-october/2007/10/10/halophilic-thermophilic-and-psychrophilic-archaea-cellular-and-molecular-adaptations-and-potential-applications.

Zhang, J., Cao, X., Xin, Y., Xue, S., and Zhang, W. (2013). Purification and characterization of a dehalogenase from Pseudomonas stutzeri DEH130 isolated from the marine sponge Hymeniacidon perlevis. *World Journal of Microbiology and Biotechnology*, *29*(10), 1791–1799.

Zhang, Y. (2008). I-TASSER server for protein 3D structure prediction. *BMC bioinformatics*, *9*(1), 1-8.