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STRUCTURAL MODEL COMPARISON OF HISTIDINE DECARBOXYLASE (HDC) OF HISTAMINE-PRODUCING BACTERIA MORGANELLA MORGANII CONSTRUCTED BY ALPHAFOLD2 AND SWISSMODEL

Komparasi Model Struktur Enzim Histidine Decarboxylase (Hdc) dari Bakteri Penghasil Histamin Produk Perikanan Morganella Morganii Yang Dikonstruksi Dengan AlphaFold2 dan Swissmodel

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ABSTRACT

Morganella morganii is one of bacteria that is capable of converting histidine to histamine in fishery products, particularly of the histidine-rich species Scobridae family. The enzyme responsible for the conversion of histidine to histamine is histidine decarboxylase (HDC), however the crystal structure of Morganella morganii HDC has not been reported. The aim of this study was to compare HDC structure models of Morganella morganii constructed de novo with AlphaFold2 and by homologous modeling with SwissModel. The overall structure of both models showed a high similarity with the superposition of α-backbone of both models (R.M.S.D.) being 0.522 Å. The active site analysis shows the position of Lys233, which can form an internal aldimine (Lys-pyridoxal-5'-phosphate) in both conservation models with Lys233 HDC from Photobacterium phosphoreum (PDB 7ERV). The main difference between the two models lies in the Asp252-Val256 residues, which form an α-helix in the AlphaFold2 model, while these residues form a loop in the SwissModel model and the P. phosphoreum HDC crystal structure. Nevertheless, the putative structure model of HDC from Tetragenococcus halophilus with a low level of amino acid sequence homology shows a different structure with an R.M.S.D reaching 1.103 Å. This research shows that modeling for HDC proteins with a high level of amino acid sequence similarity can be performed using de novo and homologous modeling methods.

Keywords: AlphaFold2, Bioinformatic, Model protein, Morganella morganii, SwissModel

ABSTRAK

Morganella morganii merupakan bakteri yang mampu mengkatalisis konversi histidin menjadi histamin pada produk perikanan seperti jenis Scobridae. Enzim yang bertanggungjawab mengonversi histidine menjadi histamin adalah histidine decarboxylase (HDC), meskipun demikian struktur kristal HDC *Morganella morganii* masih belum dilaporkan. Tujuan penelitian ini adalah melakukan perbandingan model struktur HDC dari *Morganella morganii* yang dikonstruksi *de novo* dengan AlphaFold2 dan dengan *homologous modelling* dengan SwissModel. Struktur umum kedua model menunjukkan kemiripan yang tinggi dengan hasil superimposisi karbon α kedua model (R.M.S.D) adalah 0.522 Å. Analisis sisi aktif menunjukkan posisi Lys233 yang dapat membentuk *internal aldimine* (Lys-pyridoxal-5'phosphate) pada kedua model *conserve* dengan Lys232 HDC dari *Photobacterium phosphoreum* (PDB 7ERV). Perbedaan utama kedua model terletak pada residu Asp252-Val256 yang membentuk α-helix pada model AlphaFold2, sedangkan model SwissModel dan *crystal structure* HDC *P. phosphoreum* residu tersebut membentuk *loop*. Meskipun demikian, model struktur putatif HDC dari *Tetragenococcus halophilus* dengan tingkat homologi sekuen asam amino yang rendah menunjukkan struktur yang berbeda dengan R.M.S.D mencapai 1.103 Å. Penelitian ini menunjukkan bahwa pemodelan untuk protein HDC dengan tingkat kemiripan sekuen asam amino yang tinggi dapat dilakukan dengan menggunakan metode *de novo* maupun *homologous modelling*.

Kata Kunci: AlphaFold2, Bioinformatika, Morganella morganii, Protein model, SwissModel

INTRODUCTION

Morganella morganii which can grow on fishery products containing the amino acid histidine in high concentrations is reported to be responsible for producing the biogenic amine histamine. The histamine content in fishery products is a significant problem in fishery products, especially in the Scombridae family, because the accumulation of histamine can pose serious health risks for consumers (Nevado *et al.,* 2023). Previous research has shown the presence of histamine in various fishery products, such as lemuru fish (Azzamudin *et al.,* 2024), mackarel tuna (Pertiwi *et al.,* 2020), and tuna (Witria *et al.,* 2020). Histamine production in fish is mainly caused by the activity of histidine decarboxylase (HDC), which catalyzes the decarboxylation of histidine to form the biogenic amine histamine (Lee *et al.,* 2020). HDC is one part of the decarboxylase in group II pyridoxal 5'-phosphate (PLP), catalyzing the synthesis of histamine from L-histidine with high substrate specificity (Takeshima *et al.,* 2020). Various efforts have been made to inhibit the growth of HDC-producing bacteria, including temperature control through cold chains and high pressure processing (HPP) (Lee *et al.,* 2020; Nevado *et al.,* 2023). However, specific enzyme inhibition needs to be carried out with precise targeting based on the structure of the Morganella morganii HDC protein.

The structure of the HDC protein is very important to study as a basis for strategies to mitigate the formation and accumulation of histamine in fishery products, especially Scombridae (mackerel tuna, tuna, etc.). This is because consuming fish containing high levels of histamine causes histamine poisoning, with symptoms ranging from mild such as itching to serious symptoms (Devivilla *et al.,* 2019). However, to date the crystal structure of HDC from M. morganii has not been reported, so that various efforts to study this enzyme are based on similar proteins, for example by using proteins from Photobacterium phosphoreum (PDB 7ERV) or using protein models (Tahanejad *et al.,*, 2000) . This is supported by advances in computational biology that have provided tools to predict protein structures with high accuracy. The protein structure can be obtained using homology modeling or de novo based on the characteristics of the amino acid sequence chain of a gene (Altunkulah & Ensari, 2024). Homology modeling studies of M. morganii HDCs have been previously reported compared

using the SwissModel (Tahanejad *et al.,* 2000). Recently, de novo protein modeling using AlphaFold2 was reported to have a higher level of precision (Cramer, 2021; Mirdita *et al.,* 2022). Therefore, the aim of this study was to conduct a comparative analysis of the HDC structure model from Morganella morganii built de novo with AlphaFold2, compared with the protein structure built by homology with SwissModel and the crystal structure of HDC from P. phosphoreum.

This research is expected to contribute to a deeper understanding of the relationship between protein structure and function, especially in efforts to find specific histamine inhibitors that can be applied to fishery products. Through the description and analysis of HDC structural features, the design of specific inhibitors targeting HDC from M. morganii can be constructed more precisely to prevent histamine formation in Scombridae products and other fish species susceptible to histamine contamination.

METHODS

This research was carried out in February-April 2024 at the Faculty of Fisheries and Marine Affairs, Airlangga University. The tools used are a computer, Pymol software, and an internet connection to do protein modeling.

Amino Acid Sequence Data Collection

Amino acid sequence data consists of amino acid sequences of the histidine decarboxylase (HDC) protein from *Morganella morganii* (GenBank ID: KGP44040.1), putative HDC from *Tetragenococcus halophilus* (GenBank ID: BAD81027.1), *Escherichia coli* (GenBank ID: GCQ74328.1), as well as *P. phosphoreum* (PDB ID: 7ERV).

Amino Acid Sequence Homology Analysis

Homology analysis was carried out using MEGA 11 software. Alignment analysis used ClustalW (Kumar *et al.,* 2018).

Protein Modeling

Protein modeling was carried out de novo with the online software Alphafold2 (https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb) (Bertoline *et al.,* 2023; Cramer, 2021; Mirdita *et al.,* 2022) and by using homologous modeling using SwissModel (https://swissmodel.expasy.org/) (Waterhouse *et al.,* 2018). The model obtained is then saved in the form of a PDB file for further analysis (Akdel *et al.,* 2022).

Analisis Superimposisi

Analysis of the α-carbon structural proximity of protein structures obtained with AlphaFold2 and SwissModel was carried out by superimposing the protein model with Pymol. The closeness of the structure will then be presented in the form of α-carbon distance in Root Mean Square Deviation (RMSD) in angstrom (Å) units (DeLano & Bromberg, 2004).

Active Side Prediction

Prediction of the active site was carried out by superimposing the structural model obtained with histidine decarboxylase from *P. phosphoreum* (PDB ID: 7ERV). The active site of hisitidine decarboxylase is characterized by the amino acid residue lysine which forms an internal aldimine with pyridoxal 5'-phosphate (Lys-PLP) in the P. phoshporeum 7ERV HDC protein structure.

RESULT

Homology Analysis

The amino acid sequences of histidine decarboxylase from *M. morganii, E. coli, P. phosphoreus*, and *T. halophilus* have been analyzed for close homology using ClustalW (Kumar *et al.,* 2018). The alignment results show that the HDC amino acid sequence of M. morganii has close homology to E. coli and P. phosphoreus, but is located in a separate clade from T. halophilus (Figure 1).

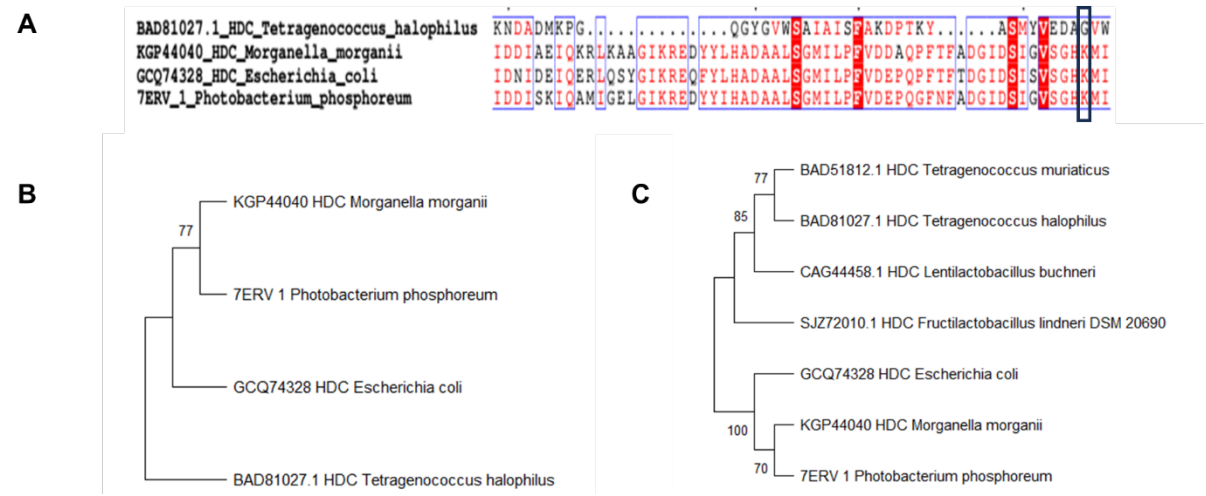

Figure 1. Close homology of histidine decarboxylase. A. Alignment of amino acid sequences of histidine decarboxylase from *M. morganii, T. halophilus, E. coli*, and *P. phosphoreum*. The black box indicates the active lysine residue based on the *P. phosphoreum* structure. B. Phylogenetic tree of histidine decarboxylase C. Phylogenetic tree with comparison of lactic acid bacteria (*T. muriaticus, T. halophilus, L. buchneri, and F. lindneri*)

The alignment results also show that there are differences in homology between HDC *Morganella morganii* and *T. halophilus*. Further analysis using the *P. phosphoreum* HDC crystal structure shows that Lys232 forms an internal aldimine bond with pyridoxal 5'-phosphate (PLP) so that it is the active lysine residue of the PLP-dependent enzyme. The alignment results show that Lys232 from HDC of *P. phosphoreum* is conserved in HDC from *E. coli* and *M. morganii*, but is not conserved in *T. halophilus*. Therefore, further analysis was carried out by comparing existing sequences with HDC from several lactic acid bacteria (LAB). The phylogenetic tree constructed using the Neigbour-Joining method with bootstrapping 1000 times shows that *M. morganii* is in the same clade as *E. coli* and *P. phosphoreum*, while all putative examples of HDC from LAB are in a separate clade.

General Structure of Protein Models

The structural model was built based on homology and *de novo*. For protein modeling using a homology approach with SwissModel, the results of homology analysis of the HDC amino acid sequence from *M. morganii* are close to the *P. phosphoreum* HDC sequence with PDB ID 7ERV. The close homology of the positions of these amino acid residues is above 80% (Figure 2A).

As for constructing a de novo HDC protein structure model, homology with a previously determined model is not required. The results of de novo modeling of the HDC protein from *M. morganii* with AlphaFold2 showed that there were five models. The similarity of the α-carbons of the five models is shown in Figure 2B.

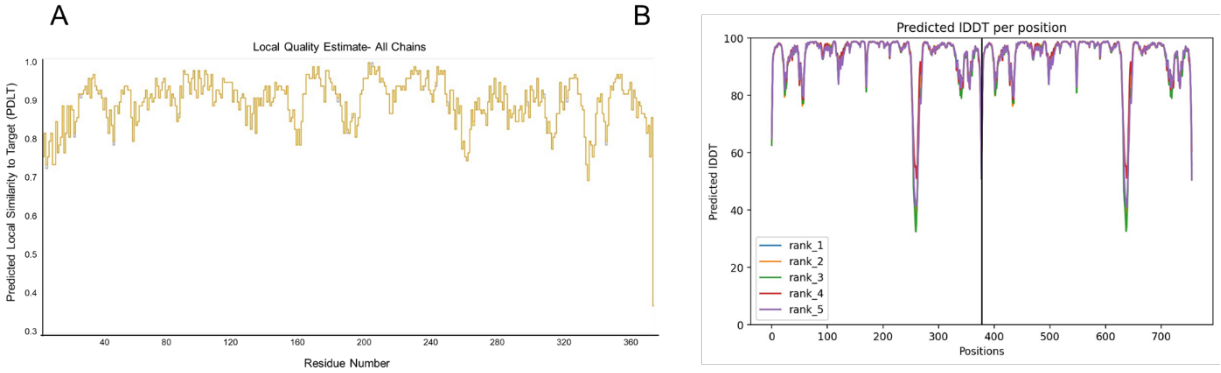

Figure 2. Amino acid homology. A. Prediction of HDC amino acid similarities from *Morganella morganii* with the homologous target (*P. phosphoreus*), B. Local Distance Difference Test (IDDT) prediction of the fifth amino acid model

Further analysis regarding the general structure showed that the HDC protein models from *M. morganii* built with SwissModel and AlphaFold2 were not much different (Figures 3A and 3B).

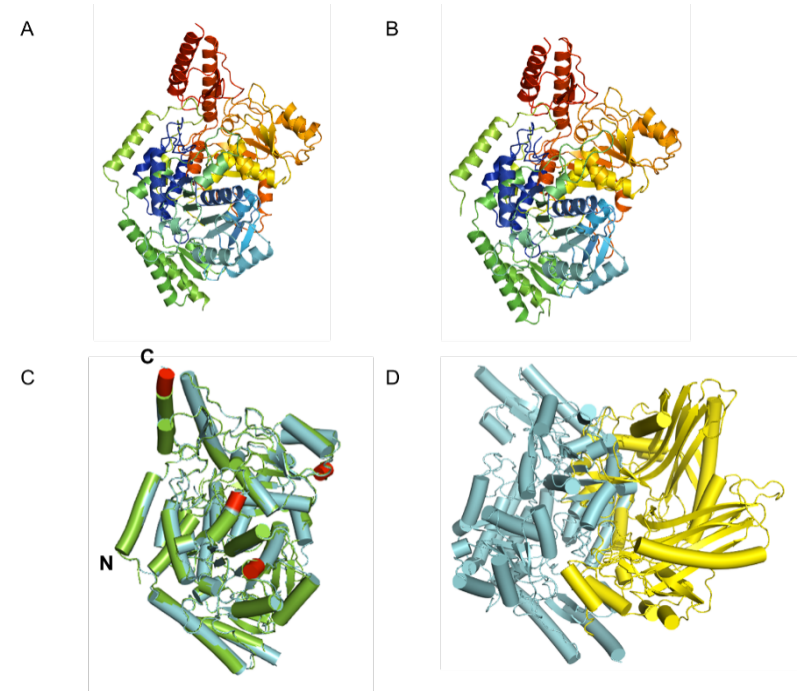

Figure 3. Structure of the model protein histidine decarboxylase from *Morganella morganii*. A. cartoon representation of *M. morganii* HDC protein model built with SwissModel, B. representation of *M. morganii* HDC built with AlphaFold2, C. Superimposition of models A and B, N-terminal indicated by the letter N and C-terminal indicated by the letter C, color red shows the difference in structure of the two models, D. Superimposition of *M. morganii* and *Tetragenococcus halophilus* HDC models.

Superimposition Analysis

Superimposition analysis of both models has been carried out using Pymol. Superimposition of protein models was carried out with structures built using SwissModel and the AlphaFold2 model ranked 1st out of 5 models built. In general, the two model structures

have a high closeness in the position of the α carbon chain with an RMSD value of 0.522 Å. Nevertheless, there are structural differences, especially at the four positions of the α-helices (Figure 3C).

Superimposition analysis of structural models of HDC from *T. halophilus* and HDC from *M. morganii* was carried out. Both models were built with AlphaFold2, and superimposition of them shows different conformations. The superimposition result between HDC from *T. halophilus* and *M. morganii* is 1.103 Å and does not form a single overlapping structure (Figure 3D).

Active Side Prediction

The active site of the histidine decarboxylase enzyme is characterized by an active lysine amino acid residue which can form internal aldimine with PLP (Eliot & Kirsch, 2004; Koper, Han *et al.,* 2022; Nevado *et al.,* 2023). To determine the active site, HDC from *P. phosphoreum* (PDB ID 7ERV) has been used. Superimposition between the HDC models SwissModel, AlphaFold2, and 7ERV shows that the active site positions of the three models overlap, so it can be assumed that the active site of the HDC enzyme from M. morganii is the same as that of *P. phosphoreum*.

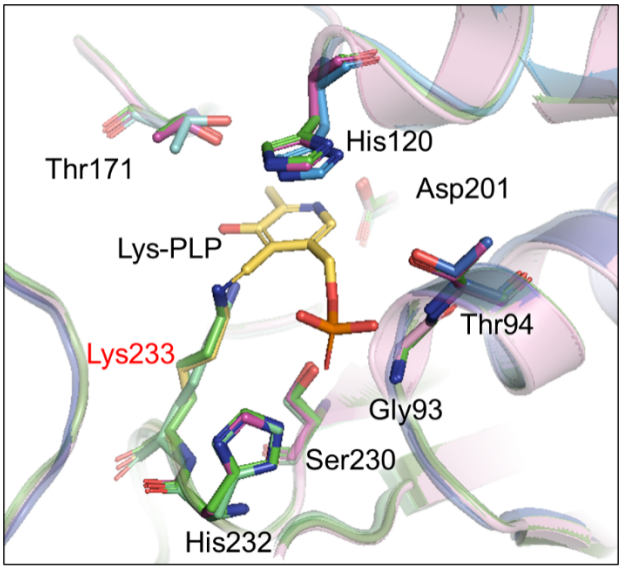

Figure 4. Prediction of the active site of histidine decarboxylase from *Morganella morganii*. Cartoon representation colors are mageta: 7ERV, green: SwissModel protein model, cyan: AlphaFold2 protein model; yellow: internal aldimine Lys233-pyridoxal-5'phosphate. Putative active residues are indicated by sticks and three-letter amino acid symbols.

Based on predictions, both models show that the active site Lys233 is in the same location as Lys233 from 7ERV. Other active sites include Thr71, Gly93, Thr94, His120, Asp201, Ser230, and His232.

DISCUSSION

Hisitidine decarboxylase is an enzyme that plays a role in catalyzing the amino acid histidine into histamine (Nevado *et al.,* 2023). In the fishing industry, histamine contamination in various products, especially Scombridae fish such as tuna and tuna, has become a major concern because histamine can have negative impacts on consumers, such as allergic reactions (Ferrante & Mercogliano, 2023). Therefore, various important efforts are made to prevent the buildup of histamine. One of the bacteria known to produce histidine decarboxylase (HDC)

and cause histamine contamination in fishery products is *Morganella morganii* (Oktariani *et al.,*, 2022). Various efforts continue to be made to prevent the growth of these bacteria, one of which is the cold chain (Hungerford, 2021). Another effort that continues to be developed is using enzyme inhibitors, but for this purpose the mechanism and structure of the protein or enzyme are needed.

The HDC of *M. morganii* was modeled using a homology approach in previous research with SwissModel (Tahanejad *et al.,* 2000). Along with technological developments, protein modeling using *de novo* approaches also continues to develop, one of which is using AlphaFold2 which shows high precision in protein modeling. AlphaFold can also be used to improve the accuracy of protein models generated from cryo-EM density maps (Cramer, 2021; Mirdita *et al.,* 2022; Pliushcheuskaya & Künze, 2023). In the homology analysis test between the selected HDC amino acid sequences, it was discovered that there were two HDC clades, namely those produced by Gram-negative bacteria, including *M. morganii*, *E. coli*, and *P. phosphoreum*, and the second clade by Gram-positive bacteria, including *T. halophilus*. *T. halophilus* is a starter culture bacteria in fish sauce production so the presence of the HDC enzyme is important to pay attention to because it has the potential to produce histamine. However, further tests showed that the HDC protein model from *T. halophilus* was very different from the HDC protein model of *M. morganii* and *P. phosphoreum* (PDB ID: 7ERV). BlastP analysis of the amino acid sequence of HDC from *T. halophilus* showed homology to HDC from trimeric pyruvoyl-dependent histidine decarboxylase from *Lactobacillus* sp. 30s (PDB ID: 1HQ6) (Schelp *et al.,* 2001). This shows that there are differences in the mechanisms and cofactors of the two proteins, where HDC from M. morganii, E. coli, and P. phosphoreum depends on PLP while HDC from *T. halophilus* depends on pyruvoyl.

Model protein yang telah dibangun, baik dengan SwissModel maupun AlphaFold2 shows the same general structure (Figure 3.). This shows that AlphaFold2 can be used to model proteins with high precision, even using only amino acid sequences, without reference structures (Akdel *et al.,* 2022). The superimposition results of the two models also show the closeness of the α-carbon position (RMSD = 0.522) with four α-helix structures that have different lengths. This is because protein modeling with AlphaFold2 refers more to the possibility of folding amino acid residues, whereas with homology modeling (SwissModel), the structure refers to a structural model that has previously been completed and stored in a database, for example in the protein data bank (PDB) (Waterhouse *et al.,* 2018).

To find out whether the two structures have the same active amino acid residue positions, a superimposition test of both models and HDC from *P. phosphoreum* was also carried out. The test results showed that the amino acid positions Thr71, Gly93, Thr94, His120, Asp201, Ser230, and His232 were located in the same position as the active amino acid residues of *P. phosphoreum* HDC (Figure 4). This shows that both models can be used for further studies regarding possible catalysis mechanisms, of course it is necessary to use more valid evidence, either by in vitro, in vivo, or in silico (for example by MD simulation). The active site is also characterized by the active lysine Lys233 which can form internal aldimine in the catalysis process by PLP-dependent enzymes (Eliot & Kirsch, 2004; Tramonti *et al.,* 2022). His120 is predicted to play a role in forming π-π interactions with the PLP ring to stabilize the position of PLP on the protein. Residues Gly93, Thr94, Ser230, and His232 are located in the phosphate group of PLP and are predicted to stabilize the position of PLP through hydrogen bonds (Eliot & Kirsch, 2004). In general, the active site of the *M. morganii* model is the same as *P. phosphoreum* (7ERV), so based on this study it can be seen that model predictions can be made using SwissModel and AlphaFold2 can be used to predict further HDC function and inhibition.

CONSLUSION

Protein models have been built using a homology approach with SwissModel and de novo with AlphaFold2. The two models have high structural similarities, both in terms of the overall structure and the location of the active residues of the HDC enzyme. Therefore, based on this study, it is known that protein models, especially HDC from *Morganella morganii*, can be prepared using both models and can be used in further studies to determine specific inhibitors and their mechanisms in silico. Of course, further studies using *in vitro* and *in vivo* approaches are also very important to be carried out to prove the results of the in *silico tests*.

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REFERENCES

Akdel, M., Pires, D. E. V., Pardo, E. P., Jänes, J., Zalevsky, A. O., Mészáros, B., Bryan, P., Good, L.L., Laskowski, R.A., Pozzati, G., Shenov, A., Zhu, W., Kundrotas, P., Serra, V.R., Rodrigues, C.H.M., Dunham, A.S., Burke, D., Borkakoti, N., Velenkar, S., Frost, A. Basquin, J., Lindorff-Larsen, K., Bateman, A., Kajava, A.V., Ovchinnikov, S., Durairaj, J., Ascher, D.B., Thornton, J.M., Davey, N.E., Stein, A., Elofsson, A., Croll, T.I., & Beltrao, P. (2022). A Structural Biology Community Assessment of AlphaFold2 Applications. *Nature Structural and Molecular Biology*, *29*(11), 1056–1067. https://doi.org/10.1038/s41594-022-00849-w

Altunkulah, E., & Ensari, Y. (2024). Protein Structure Prediction: an in-Depth Comparison of Approaches and Tools. *Eskişehir Teknik Üniversitesi Bilim ve Teknoloji Dergisi - C Yaşam Bilimleri Ve Biyoteknoloji*, *13*(1), 31–51. https://doi.org/10.18036/estubtdc.1378676

Azzamudin, A., Sipahutar, Y. H., Afifah, R. A., & Napitupulu, R. J. (2024). Pengolahan Ikan Lemuru (*Sardinella lemuru*) dalam Kaleng dengan Media Saus Tomat di PT. SY, Muncar-Jawa Timur. *Prosiding Seminar Nasional Perikanan Indonesia,* 225-244. http://dx.doi.org/10.15578/psnp.13965

Cramer, P. (2021). AlphaFold2 and the Future of Structural Biology. *Nature Structural and Molecular Biology*, *28*(9), 704–705. https://doi.org/10.1038/s41594-021-00650-1

Devivilla, S., Lekshmi, M., Kumar, S. H., Valappil, R. K., Roy, S. D., & Nayak, B. B. (2019). Effect of Sodium Hypochlorite on Biofilm-Forming Ability of Histamine-Producing Bacteria Isolated from Fish. *Journal of Food Protection*, *82*(8), 1417–1422. https://doi.org/10.4315/0362-028X.JFP-19-101

Eliot, A. C., & Kirsch, J. F. (2004). Pyridoxal Phosphate Enzymes: Mechanistic, Structural, and Evolutionary Considerations. *Annual Review of Biochemistry*, *73*, 383–415. https://doi.org/10.1146/annurev.biochem.73.011303.074021

Ferrante, M. C., & Mercogliano, R. (2023). Focus on Histamine Production During Cheese Manufacture and Processing: A review. *Food Chemistry*, *419*(March), 136046. https://doi.org/10.1016/j.foodchem.2023.136046

Hungerford, J. M. (2021). Histamine and Scombrotoxins. *Toxicon*, *201*(July), 115–126. https://doi.org/10.1016/j.toxicon.2021.08.013

Lee, Y. C., Hsieh, C. Y., Chen, M. L., Wang, C. Y., Lin, C. Saint, & Tsai, Y. H. (2020). High-Pressure Inactivation of Histamine-Forming Bacteria *Morganella morganii* and *Photobacterium phosphoreum*. *Journal of Food Protection*, *83*(4), 621–627.

https://doi.org/10.4315/0362-028X.JFP-19-267

Mirdita, M., Schütze, K., Moriwaki, Y., Heo, L., Ovchinnikov, S., & Steinegger, M. (2022). ColabFold: Making Protein Folding Accessible to All. *Nature Methods*, *19*(6), 679–682. https://doi.org/10.1038/s41592-022-01488-1

Nevado, D. L., Santos, S. D., Bastian, G., Deyta, J., Managuelod, E. J., Fortaleza, J. A., & De Jesus, R. (2023). Detection, Identification, and Inactivation of Histamine-Forming Bacteria in Seafood: A Mini-review. *Journal of Food Protection*, *26*(3), 100049. https://doi.org/10.1016/j.jfp.2023.100049

Oktariani, A. F., Ramona, Y., Sudaryatma, P. E., Dewi, I. A. M. M., & Shetty, K. (2022). Role of Marine Bacterial Contaminants in Histamine Formation in Seafood Products: A Review. *Microorganisms*, *10*(6), 1–14. https://doi.org/10.3390/microorganisms10061197

Pertiwi, R. M., Nurilmala, M., Abdullah, A., Yusfiandayani, R., & Sondita, M. F. A. (2020). Deteksi Bakteri Pembentuk Amina Biogenik pada Ikan *Scombridae* Secara Multiplex PCR. *Jurnal Pengolahan Hasil Perikanan Indonesia, 23*(2), 359-371. https://doi.org/10.17844/jphpi.v23i2.31596

Pliushcheuskaya, P., & Künze, G. (2023). Recent Advances in Computer-Aided Structure-Based Drug Design on Ion Channels. *International Journal of Molecular Sciences*, *24*(11). https://doi.org/10.3390/ijms24119226

Schelp, E., Worley, S., Monzingo, A. F., Ernst, S., & Robertus, J. D. (2001). pH-Induced Structural Changes Regulate Histidine Decarboxylase Activity in Lactobacillus 30a. *Journal of Molecular Biology*, *306*(4), 727–732. https://doi.org/10.1006/jmbi.2000.4430

Tahanejad, F. S., Naderi-Manesh, H., Habibinejad, B., & Mahmoudian, M. (2000). Homology-based Molecular Modelling of PLP-dependent Histidine Decarboxylase from *Morganella morganii*. *European Journal of Medicinal Chemistry*, *35*(6), 567–576. https://doi.org/10.1016/S0223-5234(00)00155-0

Takeshima, D., Mori, A., Ito, H., Komori, H., Ueno, H., & Nitta, Y. (2020). A Single Amino Acid Substitution Converts a Histidine Decarboxylase to an Imidazole Acetaldehyde Synthase. *Archives of Biochemistry and Biophysics*, *693*(3), 108551. https://doi.org/10.1016/j.abb.2020.108551

Tramonti, A., Ghatge, M. S., Babor, J. T., Musayev, F. N., di Salvo, M. L., Barile, A., … Contestabile, R. (2022). Characterization of the *Escherichia coli* Pyridoxal 5′-Phosphate Homeostasis Protein (YggS): Role of Lysine Residues in PLP Binding and Protein Stability. *Protein Science*, *31*(11), 1–25. https://doi.org/10.1002/pro.4471

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., … Schwede, T. (2018). Swiss-Model: Homology Modelling of Protein Structures and Complexes. *Nucleic Acids Research*, *46*(1), 296–303. https://doi.org/10.1093/nar/gky427

Witria, W., & Zainuri, M. (2021). Pengaruh Ekstrak Daun Mimba (*Azadirachta indica*) Terhadap Histamin Daging Ikan Tongkol Abu. *Jurnal Pengolahan Hasil Perikanan Indonesia, 24*(2), 209-217. https://doi.org/10.17844/jphpi.v24i2.33514