Modeling and docking studies of Keratinase from Bacillus Tequilensis MBR 25 against CYP51A of Aspergillus flavus for inhibition: Insilico studies

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ABSTRACT

Aspergillus flavus, which are common types of 'weedy' molds that are typically distributed in nature, are formed by aflatoxins. The existence of these molds does not always show that the aflatoxin levels are dangerous but shows an important danger. The molds may colonize and contaminate food before harvest or during storage, especially after prolonged exposure to high moisture or too stressful conditions such as dryness. Using 5EQB as a template for Modeller 9v7, a three-dimensional model of CYP51A from Aspergillus flavus was developed. After energy reduction, the structures of CYP51A 3D were compared with the prototype, and the final models were obtained using molecular mechanics and molecular dynamic methods. For flexible docking tests, a highly expressed CYP51A with keratinase from Bacillus tequilensis MBR 25 was used. The results showed that the LEU126, ALA153 and ILE165 binding steps of CYP51A are important determinant residues because hydrogen is closely linked to these compounds. These interactions with hydrogen bonding play an important role in stabilizing the complex.

INTRODUCTION

Keratin including quills, hide, nails, scales, horns, and hooves is a significant protein part of the creature's epidermis and related integuments. It is an insoluble protein and in light of the disulfide bond content, can be partitioned into two sorts: delicate and hard (Coulombe and Omary, 2002). Keratin is delicate and shows a low disulfide bonding content and a delicate and adaptable surface appearing in the skin. Hard keratin is available in hooks, horns and high disulfide bonds with rough, less adaptable texture (Gupta et al., 2013). Keratin primarily contain α-helices in hair or fleece, while those in quills or nails, for the most part, contain β-sheets (Bhange et al., 2016; Brandelli et al., 2010) Cross-connecting happens through disulfide bonds, hydrogen bonds and hydrophobic cooperation in keratin strands (Fakhfakh-Zouari et al., 2010). Keratin (Brandelli et al., 2010) cannot be debased by broad proteases, for example, trypsin and pepsin (Khardenavis et al., 2009; Lin et al., 1997). Keratinous squander from the meat, hide, calfskin, and butcher businesses are as of now progressively causing critical natural issues (Bressollier et al., 1999; Brockmeier et al., 2006). Some attempts were made to convert keratinous waste to modest flotsam and jetsam or protein hydrolysates using physical and synthetic drugs, which could then be used as compost applied to the feed, agriculture and corrective industries (Haddar et al., 2009).
weaken the quality and steadiness of hydrolyzed materials, prompting natural issues, for example, substantial fermentation or alkalization of the water by the waste created. Keratinases are proteins that can lyse stubborn proteins, for example, keratin specifically (Schrooyen et al., 2001). In numerous microorganisms, for example, microbes, actinomycetes and growths, keratinases have been recorded (Su et al., 2017). Also, the most very much portrayed keratinase is KerA, confined from Bacillus licheniformis PWD-1 (Zaghoul et al., 2011). Keratinases at their hydrophobic locales tie to and divide keratins. Many of the keratinases are dependent on their synergistic work, either metalloproteases or serine metalloproteases. The high keratinolytic action of microorganisms confined from keratin-rich conditions, for example, poultry plume squanders, slaughterhouse squanders, and calfskin wastes (Korniłłowicz-Kowalska et al., 2011).

Most keratinases of these strains are extracellular chemicals, intracellular, or associated with a single divider. Keratinases can be used in modern fields, such as the processing of stocks and poultry feathers, cowhide depilation, fabric cleansers, and embellishing agents because of their unique characteristics. In any case, for its application at the modern level, the vast scope creation of keratinase is significant. Diverse bacterial articulation frameworks for keratinase improvement have been utilized in past investigations (Lin et al., 1995). Bacillus subtilis can discharge local or heterologous proteins into the way of life stock successfully, making it an alluring strain for mechanical level catalyst handling. Furthermore, B. Subtilis is a sound-protein bacterium (GRAS) with a promising operation for protein requirements in food and clinical environment (Liu et al., 2014). For protein emission in B, signal peptides assume a significant job. An appropriate sign peptide could altogether help the discharge of the objective protein (Onifade et al., 1998).

In this work, to characterize the inhibitory capacity, we docked keratinase to the model CYP51A of Aspergillus flavus.

**MATERIALS AND METHODS**

**Alignment for Sequences**

Using Crustal, MAFFT, muscle and Multalin programs, various sequence alignments were carried out. The phylogenetic study with a complete length and core domain and repeat region sequences of super family keratinase proteins with different orthologues were performed, as indicated in earlier studies. Removal of the non-moderating N and C divides and the focal circle area of the individual proteins was achieved. Notwithstanding, by adjusting them to the recently decided agreement arrangement of keratinase proteins, the rationed alpha2-rehash area in every protein was resolved. The arrangements were synchronized with the muscle program and the phylogenetic tree was developed using the most extreme MEGA7 strategy with 1,000 imitated bootstrap (Altschul et al., 1990).

![Figure 1: Keratinase protein alignment showing conserved regions in bacteria.](image)

**Characterization of Proteins**

Five separate projects were anticipated to utilize the trans-layer (TM) districts in the individual Keratinase protein: TMHMM v2.0, TMMOD, Phobius, SosuSiG v1.1 and DAS-Tmfilter. For the forecast of TM geography, PROTTER v1.0 was additionally utilized. Prostate-examine, InterProScan, SMART, and NCBI protected area impact workers were utilized to construct the space piece (Altschul, 1997). Utilizing the IBS programming interface, the area design map was readied. For comparability correlation with different successions in the information base, an NCBI impact worker was utilized. Utilizing pSORT, CELLO v2.5, TargetP v1, PredSL, ngLOC and Prot-Comp workers, Within the cell, protein limitation was predicted. SignalP 4.1 was utilized to distinguish the event of the sign peptide. To gauge the isoelectric point and atomic weight, the ExPasy process pl/MW device was utilized (Brünger, 1992; Jorgensen et al., 1983).

**3D model development of CYP51AA**

From the Uniprot information base, the amino corrosive succession of CYP51A from Aspergillus flavus was gained. The protein succession from Aspergillus flavus introduced to the CYP51A receptor expectation space fishing worker. To characterize the corresponding protein structure is used as a kind of perspective by the basic local alignment search tool programming against the information base of the protein data bank, the anticipated space is looked. For
CYP51A, the gem structure isn’t accessible we chose the homology strategy for the creation of the three-dimensional structure. Modeler 9V7 with homology displaying methods was utilized to anticipate the CYP51A model (Liu et al., 2014). This program created fifty models for CYP51A and chose the least energy model for screening dependent on the low target work. Hydrogens were applied to the protein structure later on and will be balanced utilizing reenactment investigations of subatomic elements.

Structure Validation of CYP51AA Validation
The Ramachandran plot further analyses CYP51A structures with low root mean square deviation (RMSD) from studies in Molecular Dynamics, using Verify-3D to confirm the accuracy of the stereo content of protein structures with PROCHECK worker and climatic profile using the ERRAT structure evaluator (Schlenkrich et al., 1996).

The extended model MD reenactments were completed utilizing CHARMM27 power field NAMD 2.8 programming. This measurement is a different time venture with electrostatics of long-range, calculated by short-range power every two phases and each progression. In this analysis, the MD method used depends on the movement conditions of Hamilton to achieve new speeds at new positions (Kalé et al., 1999). Finally the model has been balanced for RMSD and new thermodynamic properties knowledge (Laskowski et al., 1993; Needleman, 1970).

This protein would then be able to be utilized to anticipate the dynamic site and to dock with metabolite (Eichborn et al., 2011; Daddam et al., 2020).

**Dynamic site of CYP51AA**
The conceivable CYP51A restricting locales from Cymbopogon were looked after the last model was planned dependent on the basic examination format and model develop, furthermore with CASTp worker (Daddam et al., 2020; Kusjogi et al., 2018).
Table 1: Residue percentage in Ramachandran plot areas favoured and permitted.

| Residues                                                      | Percentage |
|---------------------------------------------------------------|------------|
| Number of residues in the preferred field (~98.0% expected)   | 503 (98.4%)|
| Number of residues in the area permitted (~2.0% expected)     | 7 (1.4%)   |
| Number of residues in unfavourable areas                      | 1 (0.2%)   |

Table 2: Docking fitness scores of Keratinase with CYP51A.

| Fitness | S (hb_ext) | S (vdw_ext) | S (hb_int) | S (int) | Protein |
|---------|------------|-------------|------------|---------|---------|
| 28.25   | 8.99       | 25.49       | 0.00       | -16.91  | CYP51A  |

The CYP51A area dynamic site was anticipated utilizing the SPDBV programming dependent on the format examination structure (Kumar et al., 2017; Narendrakumar et al., 2016).

Docking Keratinase with CYP51AA

Utilizing GOLD 3.0.1 programming, a hereditary calculation utilizes a method covering three hereditary administrators, for example, relocations, transformations, and crossovers, the keratinase was docked to CYP51A (Kalé et al., 1999).

GOLD Score

For the GOLD wellness highlight score, the four boundaries, outside Vander Waals quality, interior Vander Waals power, outer H-bond, and inward hydrogen bond energy were thought of. The outside vandal score was increased to 1.375 to predict compound restriction conditions.

RESULTS AND DISCUSSION

For the correlation of different highlights of various qualities in a quality family, a few standard techniques are archived. Regarding different trademark highlights, for example, Exon-intron interaction, step intron, length, atomic weight, pl, transmembrane, cytoplasmic interaction. Circle size, cell constraints, area engineering, characteristics, and proteins of super family keratinase were investigated.

Alignment

Keratinase protein arrangement with held bacteria zones. Amino acids are shadowed in putative transmembrane parts, and amino acids are lit up, which are saved in many successions. Utilizing ClustalX programming, arrangements were made. The reports were presented in Figures 1 and 2.
CYP51A domain Homology simulation

The collected sequence comprises 420 amino acids, and there are two domains, such as CYP51A. In the BLAST run search results against PDB, 5EQB has a high degree of sequence identity along with the CYP51A domain. In CYP51A the multiple sequence alignment and antisense sequence of structurally conserved regions (SCRs) were established. 5EQB was chosen as the reference structure for CYP51A domain modeling in the following study.

![Superimposition with 5EQB of CYP51A using SPDBV tools.](image)

In the target sequence based on spatial restraint satisfaction, the SCRs, structurally variable regions (SVRs), N-termini and C-termini reference protein (5EQB) coordinates were allocated. The least energy protein was selected in Modeler 9v7 out of fifty developed PDBs. Rotamers were used to set all the model protein side chains. The final stable structure of the CYP51A protein is, as shown in Figure 3.

![The active site representation of the CYP51A receptor pockets displays the highest area, volume (red color).](image)

With the help of SPDBV, it shows that the CYP51A domain contains 16 helixes and four sheets. The low energy structure obtained by NAMD, with low RMSD (Root Mean Square Deviation) is shown in Figure 4. By verify 3D graph, the structure was further analyzed, the results were shown in Figure 5. The total scores indicate an appropriate protein environment.

CYP51A Domain Validation

Protein validation using Ramachandran plot calculations was performed after the refinement phase based on PROCHECK Software Figure 6. Table 1 is tabulated with the distribution of non-glycine, non-proline amino acids in the Ramachandran plot. The difference from the standard CYP51A dictionary for the RMSD was -1.25 and -0.85 Å. For angles of covalence and covalent bonds. A total of 98.4% of CYP51A residues were in favored and accepted areas. The overall PROCHECK G-factor CYP51A was 1.74 and Verified 3D was excellent.

5EQB template superimposition with domain CYP51A

The structural superposition of the 5EQB prototype and CYP51A and the mean root square deviation between the template and the final refined models is 0.49 Å. To predict active site based on the structural similarity feature, this surrounding of protein and template structures Figure 7.

![Docking keratinase studies with CYP51AA.](image)

Active site recognition of CYP51AA identification

The possible CYP51AA binding sites have been searched based on the structural comparison of the template with model construct, and GLN8, LYS9, VAL10, TRP11, GLU48, ASN49, also found with the CASTp server and with the residues Figure 8.

Inhibitory keratinize experiments on CYP51A

Potential binding sites for CYP51A were identified using the CASTp server in this study the largest binding site in volume and size was selected from these estimated binding sites, and the amino acid was chosen for studies in this field. The metabolite proteins with GOLD 3.0.1 have been injected into CYP51A and all CYP51A docking solutions have been rated for GOLD health. The results of the docking showed that metabolite proteins are an active fitness inhibitor of CYP51A. Metabolite proteins display the strongest docking effects, indicating inhibitory activity Figure 9. In binding studies, a hydrogen bond was documented to consist of metabolite proteins CYP51A with a bonding distance of 2.54 Å. In the CYP51A inhibitor complex, atomic metabolite hydrogen was
formed by a 28.25 KJ/mol hydrogen bond with a GLU48 oxygen atom (O8). Table 2 displays the best inhibitors of CYP51A and docking fitness values.

**CONCLUSIONS**

We have developed the 3D model of domain CYP51A, using homology modeling technique, and obtained a refined model after minimizing the energy. The final refined models have been tested by PROCHECK and the results indicate that the models are accurate. The stable structure of CYP51A has also been used to dock with *Bacillus tequilensis* MBR 25 keratinase. Docking results show that the CYP51A which is the main amino acid residue plays an important role in preserving functional conformation and is directly involved in the binding of the donor substrate. Interaction between domain and keratinase which is proposed in this study is useful to understand the possible method of the field and the binding of the inhibitor. As it is well-known that the structure and function of biological molecules play a vital role in hydrogen bonds. In this analysis, LEU 126, ALA153 and ILE165 in CYP51A were found to be essential for intense interaction between the hydrogen and the inhibitors. These are best known in this area and can be important for structural integrity or the protection of the hydrophobicity of the pocket inhibitor.

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**Conflict of Interest**

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**REFERENCES**

Altschul, S. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25(17):3389–3402.

Altschul, S. F., et al. 1990. Basic local alignment search tool. *Journal of Molecular Biology*, 215(3):403–410.

Bhave, K., et al. 2016. Ameliorating effects of chicken feathers in plant growth promotion activity by a keratinolytic strain of Bacillus subtilis PF1. *Bioresources and Bioprocessing*, 3(1):13.

Brandelli, A., et al. 2010. Biochemical features of microbial keratinases and their production and applications. *Applied Microbiology and Biotechnology*, 85(6):1735–1750.

Bressollier, P., et al. 1999. Purification and Characterization of a Keratinolytic Serine Proteinase from Streptomyces albidoflavus. *Applied and Environmental Microbiology*, 65(6):2570–2576.

Brückmeier, U., et al. 2006. Systematic Screening of All Signal Peptides from Bacillus subtilis: A Powerful Strategy in Optimizing Heterologous Protein Secretion in Gram-positive Bacteria. *Journal of Molecular Biology*, 362(3):393–402.

Brünger, A. T. 1992. X-PLOR: version 3.1: a system for x-ray crystallography and NMR. Yale University Press. pages: 400.

Coulombe, P. A., Omary, M. B. 2002. Hard and soft principles are defining the structure, function and regulation of keratin intermediate filaments. *Current Opinion in Cell Biology*, 14(1):110–122.

Daddam, J. R., et al. 2014. Molecular docking and P-glycoprotein inhibitory activity of Flavonoids. *Interdisciplinary Sciences: Computational Life Sciences*, 6(3):167–175.

Daddam, J. R., et al. 2020. Designing, docking and molecular dynamics simulation studies of novel cloperastine analogues as anti-allergic agents: homology modeling and active site prediction for the human histamine H1 receptor. *RSC Advances*, 10:4745–4754.

Eichhorn, J. V., et al. 2011. PROMISCUOUS: a database for network-based drug-repositioning. *Nucleic Acids Research*, 39(1):1060–1066.

Fakhfakh-Zouari, N., et al. 2010. Application of statistical experimental design for optimization of keratinases production by Bacillus pumilus A1 grown on chicken feather and some biochemical properties. *Process Biochemistry*, 45(5):617–626.

Gupta, R., et al. 2013. Biotechnological applications and prospective market of microbial keratinases. *Applied Microbiology and Biotechnology*, 97(23):9931–9940.

Haddar, H. O., et al. 2009. Biodegradation of native feather keratin by Bacillus subtilis recombinant strains. *Biodegradation*, 20(5):687–694.

Jorgensen, W. L., et al. 1983. Comparison of simple potential functions for simulating liquid water. *The Journal of Chemical Physics*, 79(2):926–935.

Kalé, L., et al. 1999. NAMD2: Greater Scalability for Parallel Molecular Dynamics. *Journal of Computational Physics*, 151(1):283–312.

Khardenavis, A. A., et al. 2009. Processing of poultry feathers by alkaline keratin hydrolyzing enzyme
from Serratia sp. HPC 1383. Waste Management, 29(4):1409–1415.

Korniłowicz-Kowalska, et al. 2011. Biodegradation of keratin waste: Theory and practical aspects. Waste Management, 31(8):1689–1701.

Kotha, P., et al. 2015. Modelling simulation phylogenetics of leukemia FMS tyrosine kinase 3 (FLT3). Online Journal of Vet Research, 16(1):8–17.

Kotha, P. N., et al. 2017. Molecular dynamics and protein interaction studies of lipopeptide (Iturin A) on α- amylase of Spodoptera litura. Journal of Theoretical Biology, 415:41–47.

Kurjogi, M., et al. 2018. Computational Modeling of the Staphylococcal Enterotoxins and Their Interaction with Natural Antitoxin Compounds. International Journal of Molecular Sciences, 19(1):133.

Laskowski, R. A., et al. 1993. PROCHECK: a program to check the stereochemical quality of protein structures. Journal of Applied Crystallography, 26(2):283–291.

Lin, X., et al. 1995. Nucleotide sequence and expression of kerA, the gene encoding a keratinolytic protease of Bacillus licheniformis PWD-1. Applied and environmental microbiology, 61(4):1469–1474.

Lin, X., et al. 1997. Expression of the Bacillus licheniformis PWD-1 keratinase gene in B. subtilis. Journal of Industrial Microbiology and Biotechnology, 19(2):134–138.

Liu, B., et al. 2014. Comparative analysis of bacterial expression systems for keratinase production. Applied Biochemistry and Biotechnology, 173(5):1222–1235.

Narendrakumar, P., et al. 2016. In silico and in vitro studies of fungicidal nature of lipopeptide (Iturin A) from Bacillus amyloliquefaciens RHNK 22 and its plant growth promoting traits. Indian Phytopathology, 69(4s):569–574.

Needleman, S. B. 1970. A general method applicable to the search for similarities in the amino acid sequence of two proteins. Journal of Molecular Biology, 48(3):443–453.

Onifade, A. A., et al. 1998. A review: Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. Bioresource Technology, 66(1):1–11.

Rayalu, D. J., et al. 2012. Homology modeling, active site prediction, and targeting the anti hypertension activity through molecular docking on endothelin – B receptor domain. Bioinformation, 8(2):81–86.

Schlenkrich, M., et al. 1996. An empirical potential energy function for phospholipids criteria for parameter optimization and applications. Biological Membranes, pages 31–81.

Schrooyen, P. M., et al. 2001. Partially Carboxymethylated Feather Keratins. Journal of Agricultural and Food Chemistry, 49(1):221–230.

Su, C., et al. 2017. A novel alkaline surfactant stable keratinase with superior feather degrading potential based on library screening strategy. International Journal of Biological Macromolecules, 95:404–411.

Zaghloul, T. I., et al. 2011. Key determinants affecting sheep wool biodegradation directed by a keratinase-producing Bacillus subtilis recombinant strain. Biodegradation, 22(1):111–128.