Molecular Modeling of Cloned *Bacillus subtilis* Keratinase and Its Insinuation in Psoriasis Treatment Using Docking Studies

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Abstract Present study demonstrated the expression of cloned *Bacillus subtilis* RSE163 keratinase gene and in silico binding affinities of deduced protein with psoriasis topical drugs for systemic absorption and permeation through skin. The *ker* gene expressed in *E. coli* showed significantly higher keratinase activity 450 ± 10.43 U representing 1342 bp nucleotides encoding 447 amino acids with molecular weight of 46 kDa. The modeled structure was validated using ramachandran's plot showing 305 residues (84.3%) in most favoured region. Docking studies using extra precision (XP) method of Glide showed optimum binding affinities with the drugs Acitretin (− 39.62 kcal/mol), Clobetasol propionate (− 37.90 kcal/mol), Fluticasone (− 38.53 kcal/mol), Desonide (− 32.23 kcal/mol), Anthralin (− 38.04 kcal/mol), Calcipotriene (− 21.55 kcal/mol) and Mometasone (− 28.40 kcal/mol) in comparison to other psoriasis drugs. The results can further be correlated with in vitro enzymatic experiments using keratinase as an effective drug mediator through skin to serve the unmet need of industries.

Keywords Keratinase · Affinity · Docking · Structural modeling · Psoriasis

Introduction

Keratinases are unique group of proteolytic enzymes adept to hydrolyze different keratin substrates viz. α -keratins (hair, hooves, nail etc.) and β -keratins (Feather and silk fibrions, β -amyloid) into simple polypeptides and amino acids [1]. Capricious activity of keratinase towards different keratin substrates is due to their extracellular secretion from microorganisms like bacteria, fungi and actinomycetes [2]. Since the skin and nails composed of keratins, keratinase are thought to be alternative ungual enhancers in hyperkeratosis (psoriasis, nail disorders) conditions which hydrolyze the keratin and thus can enhance the permeability and efficacy of topical drugs [3]. Psoriasis is a common inflammatory skin disease personified by keratinocyte, hyperproliferation, dedifferentiation, neoangiogenesis and inflammation affecting 2–3% of the world population. Presently a broad range of treatment modalities are available for psoriasis viz. topical treatments, light therapy and systemic medications [4]. Topical medications considered to be the first line of defense, generally used to treat the mild to moderate psoriasis to decelerate the unnecessary cell reproduction and diminish the inflammation associated with this [5]. A range of ointments, gels, pastes, creams and scalp solutions containing anthralin, calcipotriene, calcitriol, tazarotene and salicylic acid etc. were used as medication. Psoriasis lesions are noticeably thickened and thinned epidermis with heterogeneity in the skin morphology leads to variability in drug permeation and their systemic absorption [6]. We are

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working on the *Bacillus subtilis* keratinase since last 6 years and found promising hydrolyzing proficiency towards keratin which would be a subject of substantial scientific interest towards psoriasis treatment [2, 7]. Present study demonstrated the expression of *ker* gene in *E. coli* and in silico binding affinities of deduced protein with psoriasis topical drugs using docking studies.

Materials and Methods

Bacillus subtilis RSE163 (NCBI Accession No. JQ887983) procured from the laboratory culture collection was used for the present study.

Molecular Cloning and Expression of *ker* Gene

Bacillus subtilis RSE163 (NCBI Accession No. JQ887983) *ker* gene primers were designed based on high degree of sequence homology published for the keratinase of *Bacillus* strains. Cloning sites of EcoRI were introduced in forward primer (5'-GGTACC-GAATTCGAGTCTCTACGGAAATAGC-3') and BamHI in reverse (5'-TCTAGAGGATCCGA-GAATGCGCCGGAACATCAGG-3'). Standard PCR were performed using 100 ng of genomic DNA in 100 µl reaction containing phusion mix and 5 µM primers in master cycler (Eppendorf, Germany) programmed for 31 cycles, with an initial denaturation temperature at 94 °C for 5 min and each cycle with 94 °C for 30 s, annealing at 65 °C for 30 s, extension at 72 °C for 1 min and final extension at 72 °C for 5 min. PCR product were run at 1.2% agarose and purified from Qiagen gel extraction kit and sent for sequencing. Obtained sequence of keratinase (*ker*) gene from *Bacillus subtilis* were submitted in NCBI and Accession no. has been taken. For molecular cloning this amplified product was digested with EcoRI and BamHI (New Englands Biolabs) and inserted into the corresponding sites of pSPORT expression vector to obtain the recombinant plasmid through ligation reaction using enzyme T4 ligase. Ligated DNA (20 µl) was added to 0.1 ml freshly prepared competent cell having transformation efficiency of 3.2×10^5 cfu/µg DNA. Ligated DNA was transformed into *E. coli* cells using heat shock method and sample were incubated for 1 h at 37 °C. After incubation sample were plated on Luria agar plate containing ampicillin (100 µg/ml) and incubated overnight at 37 °C, putative *ker* clones were again streaked on appropriate antibiotic containing plate and incubate at 37 °C for 16–20 h. Positive clones were monitored for extracellular expression at 37 °C by estimating the keratinase activity.

Assay of Keratinolytic Activity

The keratinolytic activity of strain was determined by using the cell free extract of feather media as per the protocol published in our previous paper [7].

Sequence Analysis

With the help of online translate tool of ExPASy (<http://web.expasy.org/translate>) keratinase protein sequence was obtained from the experimentally determined *ker* gene of *Bacillus subtilis*. The deduced amino acids were analyzed using NCBI protein blast. Blast searches the non redundant protein sequences database and hits with an e-value (< 0.0005) and sequence identity ($> 30\%$) were considered as matches. Alignment of protein sequences were carried out using CLUSTALW program at the European Bioinformatics Institute server (<http://www.ebi.ac.uk/clustalw>). The FingerPRINTScan tool was used for the identification of the closest family on the basis of catalytic sites and matching PRINTS sequence motif fingerprints and in a protein sequence [8].

Structure Prediction and Validation of Keratinase Enzyme

A PDB BLAST search of the protein sequence was carried out for selection of templates based on sequence identity and similarity. Iterative implementation of the Threading Assembly Refinement (I-TASSER) program using two templates with PDB code (protein data bank): 3WHI (with 95% identity) and 1MEE (with 99% identity) belonging to subtilisin family of hydrolase enzymes were used for 3D modelling [9]. Five models were generated and the best model with a confidence score (C-score which is a measure of quality of the predicted model) of -2.24 and potential energy of -1350.56 kcal/mol was selected for further study. The predicted structure was further refined by energy minimization with Optimized Potentials for Liquid Simulations 2005 (OPLS 2005) force field to alleviate steric clashes [10]. Energy Minimization was carried out until average root mean square deviation of $0.3 \text{ \AA}$ was reached for non-hydrogen atoms. The stereo chemical analysis of the predicted structure was carried out by PROCHECK [11]. Further, PDB sum was used to generate the secondary structure information of the protein. Finally, the Protein Preparation workflow in the Maestro interface was used on the 3D model of the *Ker* protein to add *N*-acetyl and *N*-methyl amide capping groups to the N-terminus and C-terminus respectively. A RMSD of $0.30 \text{ \AA}$ was set for the constrained refinement of the receptor mode. The heavy atoms were constrained in the first minimization in order to allow free rotation of the

hydrogen. Subsequently, minimizations were performed by decreasing the constraints on the heavy atoms.

Binding Pockets

Site-Map module of Maestro was used for binding site predictions in the keratinase enzyme [12]. For the identification of possible binding sites, physical descriptors such as size, degree of enclosure/exposure, hydrophobic/-philic character, tightness and hydrogen-bonding possibilities were considered.

Docking Studies of Keratinase with Psoriasis Topical Drugs

The molecular docking studies were carried out between the 3D model of keratinase and topical drugs that are used in psoriasis treatment belonging to all categories: Corticosteroids (Clobetasol propionate, Fluticasone, Desonide and Mometasone), Vitamin D analogues (Calcipotriol), Retinoids (Tazarotene, Acitretin) and other topical agents (Psoralen, Allantoin, Resorcinol, Anthralin and Methoxsalen). These drugs were modeled using build application of Maestro 9.3. The geometry of these drugs was optimized by molecular mechanics using impact in a dynamic environment with standard TIP4P water model. For energy minimization Polak-Ribier conjugate gradient and Truncated Newton conjugate gradient algorithms were performed using Optimized Potentials for Liquid Simulations 2005 (OPLS 2005) force field. 0.01 Å RMS gradient was used as the convergence threshold. Conformational models of the drugs were generated by ligprep with Epik at 7.0 ± 2.0 pH units to expand protonation and tautomeric states. Extra precision (XP) method called Glide (Grid-based Ligand Docking with Energetics) was used for carrying out the protein–ligand docking [13]. The receptor grid generation for docking was done using the centroid of selected predicted active site residues as well as blind docking. The different conformations of the compounds were docked flexibly and 1000 poses per compound were generated. Schrodinger's software suite was used for the analysis of complexes, poses and binding affinities between the receptor and ligands. The best poses were chosen based on their binding energies and G-score (Glide score).

Results and Discussion

Cloning and Expression of *ker* Gene

Keratinase (*ker*) gene (NCBI Accession no. KM494493) belongs to *Bacillus subtilis* RSE163 was successfully

amplified by using gene specific primers engender the 1.3 kb product using genomic DNA (Fig. S1). The constructed vector represent as pSPORT *ker* gene was transformed into *E. coli* Mach 1. Positive clones were monitored for extracellular expression at 37 °C showed significantly higher keratinase activity 450 ± 10.43 U compare to wild type strain [2]. Cloning and over expression of keratinase have been carried out to meet the demand of enzyme industry in various fields [14].

Sequence Analysis

Keratinase (*ker*) gene sequence analysis revealed the presence of 1342 bp nucleotides encoding 447 amino acids with predicted molecular weight of 46 kDa (Fig. S2). The sequence which has been used for the further study was chosen from amino acid 61–423 (362 amino acids) obtained from longest open reading frame. Determination of nucleotide sequence of *ker* gene provided the phylogenetic study with its decoded structure–function relationships [15]. The amino acid sequence of the *ker* gene was subjected to NCBI protein blast and results showed 97% similarity with *ker C Bacillus subtilis*. The blast analysis revealed the presence of subtilisin region belongs to peptidase S8 family (Peptidase subtilisin S8HPTWTNAQVR). The S8 family has an Asp/His/Ser catalytic triad similar to trypsin-like proteases. Serine acts as a nucleophile, aspartate as an electrophile, and histidine as a base which is conserved in all previously isolated keratinase belong to *Bacillus* strains [14].

Protein BLAST found the Chain A of crystal structure of unautoprocessed form of 1s-1m inserted Pro-subtilisin E from *Bacillus subtilis* (PDB ID: 3WHI) as the closest match; with 80% query coverage and sequence identity of 95%. While in other results chain A of the complex between subtilisin from a mesophilic bacterium and the leech inhibitor Eglin-C from *Bacillus pumilus* (PDB ID: 1MEE), with 62% query coverage and sequence identity of 99% and finally chain A crystal structure of subtilisin-propeptide complex from *Bacillus subtilis* (PDB ID: 1SCJ), with 62% query coverage and sequence identity of 99%. The e-value in all the three results was zero.

The multiple sequence alignment of the keratinase enzyme with other subtilisin from the *Bacillus* family is shown in Fig. 1. The FingerPRINTScan tool was used to identify the closest matching PRINTS sequence motif fingerprints in the keratinase enzyme [16]. The highest scoring fingerprints were deduced from subtilisin family with an e-value of $3.521983e-23$. A total of three motifs were identified, the sequences that are members of family S8 have a catalytic triad in order of Asp, His and Ser, whereas the order is different in other families S1, S9 and S10. In keratinase enzyme the active site or catalytic triad is well

Fig. 1 Multiple sequence alignment of the experimentally determined *ker* enzyme with other subtilisin from the *Bacillus* family. The PDB codes of the other enzymes are listed. Dark blue colour indicates the identical residues, medium blue colour indicates strong similarity

conserved and marked with red stars. The S8 family is divided into two subfamilies S8A and S8B. In subfamily S8A, the active site residues frequently occurs in the motifs Asp-Thr/Ser-Gly that is similar to the motif in families of aspartic endopeptidases in clan AA, His-Gly-Thr-His and Gly-Thr-Ser-Met-Ala-Xaa-Pro. In subfamily S8B, the catalytic residues frequently occur in the motifs Asp-Asp-Gly, His-Gly-Thr-Arg and Gly-Thr-Ser-Ala/Val-Ala/Ser-Pro. The analysis revealed the presence of DSG that is Asp 179 to Gly 181, HGTH that is His 211 to His 214 and GTSMaXP from Gly 366 to Pro 372. This reveals that the *ker* enzyme belong to the subfamily S8A of S8.

Structure Prediction and Validation

Comprehension of 3D structure of an enzyme is a prerequisite for its structure-based design. Till date only a few crystal structures of enzymes are reported. Kim et al. [17] reported a 1.7 Å resolution crystal structure of *Fervidobacterium pennivorans* keratinase (fervidolysin) showed high homology with subtilisin-like serine proteases. Protein sequences with unknown structure information can be dependably modeled via protein 3D structure prediction software which facilitates the acquaintance relationship between protein sequence, structure, dynamics and function which is used to discover the interaction of protein with other molecules or ligands [18].

I-TASSER generated five models (Model 1 Model 2, Model 3, Model 4, and Model 5) for 362 residues of the

and light blue colour indicates the weak similarity. Conserved motifs across the subtilisin enzyme are shown in boxes, while the catalytic triad residues are marked with a red “*” (colour figure online)

hypothetical keratinase protein based on their cluster density [9]. The templates identified for multiple sequence alignment were: 3WHI, 1MEE, 4JP8, 1S01 with identity ranging from 43 to 100%. The normalized Z-score for these models varied between 5.63 and 2.58 indicating, a high alignment between the keratinase protein sequence and templates. The C-scores (Confidence scores) for the different models were: Model 1: – 2.24, Model 2: – 2.41, Model 3: – 2.31, Model 4: – 2.98 and Model 5: – 2.57. Model 1 with highest C-score was chosen for further since a high C-score value signifies a model with a high confidence and vice versa (Fig. 2). Topology and secondary structure of the predicted keratinase enzyme revealed its units, which comprises of sixteen stranded beta sheets (A–E) and twelve alpha helices (H1–H12), whereas motifs represented as beta (β) and gamma (γ) turn along with beta hairpin (Ψ) (Fig. S3 and S4). Validation of the model has been performed with stereochemical standards using Ramachandran plot, generated from PROCHECK program (Fig. S5). Ramachandran’s plot calculations revealed 305 residues occurred in most favoured region, accounts for 84.3% of the total residues which clearly signified the stability and goodness of the model for in silico studies [19].

Binding Pocket

Keratinase enzyme revealed four possible ligand binding sites in perspective of ligand donor, ligand acceptor and

Fig. 2 Hypothetical structure of keratinase (Model-1) elucidated from I-TASSER programme based on cluster density

hydrophobic groups (blue, red, and yellow respectively) [12]. These pockets were compared with the active site of the other keratinase enzymes which share high sequence identity with the *Bacillus subtilis* RSE163 keratinase enzyme. Fig. S6 represented the site 1 of the enzyme which is highly conserved with a site score of 0.996 and volume of 814.625 Å³.

Docking Studies of Keratinase with Psoriasis Topical Drugs

Docking results showed high docking score of keratinase with drugs acitretin, clobetasol propionate, fluticasone, desonide, Anthralin, calcipotrene and mometasone clearly demonstrated their binding affinity. Docking score and energy of the complexes are represented in Table 1. Molecular docking is generally used to learn the drug-receptor interaction to envisage the affinity and activity of the small molecule to their protein [20]. The model energy score (E-model) which combines the glide score, the excess internal energy and non bonded interaction energy of the generated ligand conformation and the glide score were used to rank the docked conformation of the ligands with the protein (fig. S7). The best binding conformation with the lowest G-Score was observed in the case of acitretin (fig. S8a). Methoxsalen showed least affinity for keratinase enzyme. Both the retinoids tazarotene and acitretin bind to keratinase enzyme with the same orientation and both the compounds make hydrogen bonds with Gln 108. The

acitretin shows better binding affinity as it is involved in additional hydrogen binding and also forms a salt bridge with Lys 79. Out of the four corticosteroids used for the study clobetasol propionate, fluticasone and desonide have similar glide scores. Both clobetasol propionate and fluticasone interact with the same orientation and the cyclohexadienone moiety forms hydrogen bond with the backbone of Ile 58, and also involved in hydrophobic interactions with desonide. In addition clobetasol propionate is involved in additional two hydrogen bonds one with the backbone of Ala 58 and another with Ser 248, whereas, fluticasone involved with Thr 246 only (Fig. S8b). Desonide binds with a different orientation and forms hydrogen bonds with Ser 248 and Asp 244. Mometasone though shares the same parent moiety with the other corticosteroids, an additional furoic acid moiety provides a steric hindrance for the compound to move inside the binding cavity which might account for a low binding affinity. Vitamin D analog calcipotriol or calcipotriene made two hydrogen bonds; the dihydro cyclohexanone moiety forms hydrogen bond with Gln 108 whereas the hydroxyl attached to methylcyclopropane made hydrogen bond with Ser 248. Both the hydroxyl and a keto oxygen attached to each the three benzenes in anthralins formed three hydrogen bonds. The hydroxyls formed hydrogen bonds with backbone of Ser 248 and side chain of Asp 244 and the keto oxygen with Trp 253. A $\pi - \pi$ stacking of the phenol was also observed with Phe 59. These interactions of anthralin with the keratinase enzyme account for a high binding affinity of -38.04 kcal/mol. The main contributing factors for a good binding affinity with anthralin are the coulombic and vander wall energies of -9.96 and -23.63 kcal/mol. Other psoriasis drugs Allantoin, Psoralen, Resorcinol and methoxsalen resulted in a poor binding affinity with the keratinase enzyme. Docking studies revealed the binding affinity of keratinase with psoriasis drugs at non catalytic site will be further explored in vitro to check the probability of combination of keratinase with topical drugs in improving its permeability and increasing efficacy. Docking of *Stenotrophomonas* sp. keratinase and *Bacillus licheniformis* keratinase has been reported in order to enhance their catalytic efficiency [21, 22].

Conclusion

The nucleotide sequence of the *Bacillus subtilis* *ker* gene were determined and successfully expressed in *E. coli* using pSPORT expression vector will be the basis of their large scale production for its scientific applications in the near future. In silico studies of deduced protein showed potential binding affinities with psoriasis drugs clearly

Table 1 Structures, docking score and energy of the complexes

Drugs		Structure	Docking Score	Energy of the model (Kcal/mol)
Vitamin analogs	D			
	Calcipotriol		−4.56769	−21.55
Retinoids	Tazarotene		−4.10318	−34.11
	Acitretin		−5.19984	−39.62
Corticosteroids	Clobetasol propionate		−4.91077	−37.90
	Fluticasone		−4.83688	−38.53
	Desonide		−4.73229	−32.23
	Mometasone		−4.01794	−28.40
Other agents	Allantoin		−3.59965	−26.43
	Psoralen		−3.89938	−21.34
	Resorcinol		−3.45811	−21.50
	Methoxsalen		−3.02786	−26.26
	Anthralin		−4.6287	−38.04

demonstrated its utility in improvement of permeability and efficacy of drugs through skin.

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References

- Brandelli A, Daroit DJ, Riffel A (2010) Biochemical features of microbial keratinases and their production and applications. *Appl Microbiol Biotechnol* 85:1735–1750. doi:[10.1007/s00253-009-2398-5](https://doi.org/10.1007/s00253-009-2398-5)
- Gupta S, Singh R (2014) Hydrolyzing proficiency of keratinases in feather degradation. *Indian J Microbiol* 54:466–470. doi:[10.1007/s12088-014-0477-5](https://doi.org/10.1007/s12088-014-0477-5)
- Mohorcic M, Torkar A, Friedrich J, Kristl J, Murdan S (2007) An investigation into keratinolytic enzymes to enhance ungual drug delivery. *Int J Pharm* 332:196–201. doi:[10.1016/j.ijpharm.2006.09.042](https://doi.org/10.1016/j.ijpharm.2006.09.042)
- Kircik LH, Del Rosso JQ (2009) Anti-TNF agents for the treatment of psoriasis. *J Drugs Dermatol* 8:546–559
- Uva L, Miguel D, Pinheiro C, Antunes J, Cruz D, Ferreira J, Filipe P (2012) Mechanisms of action of topical corticosteroids in psoriasis. *Int J Endocrinol* 2012;2012(2012):16. Art id:561018. doi:[10.1155/2012/561018](https://doi.org/10.1155/2012/561018)
- Valdman-Grinshpoun Y, Ben-Amitai D, Zvulunov A (2012) Barrier-restoring therapies in atopic dermatitis: current approaches and future perspectives. *Dermatol Res Pract* 2012;2012:6. Art id:923134. doi:[10.1155/2012/923134](https://doi.org/10.1155/2012/923134)
- Gupta S, Singh SP, Singh R (2015) Synergistic effect of reductase and keratinase for facile synthesis of protein coated gold nanoparticles. *J Microbiol Biotechnol* 25:612–619. doi:[10.4014/jmb.1411.11022](https://doi.org/10.4014/jmb.1411.11022)
- Attwood TK, Coletta A, Muirhead G, Pavlopoulou A, Philippou PB, Popov I, Romá-Mateo C, Theodosiou A, Mitchell AL (2012) The PRINTS database: a fine-grained protein sequence annotation and analysis resource its status in 2012. *Database*. doi:[10.1093/database/bas019](https://doi.org/10.1093/database/bas019)
- Yang J, Yang Z (2015) I-TASSER server: new development for protein structure and function predictions. *Nucleic Acids Res* 43:W174–W181. doi:[10.1093/nar/gkv342](https://doi.org/10.1093/nar/gkv342)
- Singh KD, Muthusamy K (2013) Molecular modeling, quantum polarized ligand docking and structure-based 3D-QSAR analysis of the imidazole series as dual AT1 and ETA receptor antagonists. *Acta Pharmacol Sin* 34:1592–1606. doi:[10.1038/aps.2013.129](https://doi.org/10.1038/aps.2013.129)
- Kulkarni PA, Devarumath RM (2014) *In silico* 3D-structure prediction of SsMYB2R: a novel MYB transcription factor from *Saccharum spontaneum*. *Indian J Biotechnol* 13:437–447
- Kesharwani RK, Misra K (2011) Prediction of binding site for curcuminoids at human topoisomerase II α protein; an *in silico* approach. *Curr Sci* 101:1060–1065
- Tewatia P, Malik BK, Sahi S (2012) Identification of novel β 3-adrenoceptor agonists using energetic analysis, structure based pharmacophores and virtual screening. *Comb Chem High Throughput Screen* 15:623–640. doi:[10.2174/138620712802650559](https://doi.org/10.2174/138620712802650559)
- Jaouadi NZ, Rekik H, Badis A, Trabelsi S, Belhouli M, Yahiaoui AB, Aicha HB, Toumi A, Bejar S, Jaouadi B (2013) Biochemical and molecular characterization of a serine keratinase from *Brevibacillus brevis* US575 with promising keratin-biodegradation and hide-dehairing activities. *PLoS ONE* 8:e76722. doi:[10.1371/journal.pone.0076722](https://doi.org/10.1371/journal.pone.0076722)
- Frazer KA, Elnitski L, Church DM, Dubchak I, Hardison RC (2003) Cross-species sequence comparisons: a review of methods and available resources. *Genome Res* 13:1–12. doi:[10.1101/gr.222003](https://doi.org/10.1101/gr.222003)
- Narayanan A, Chen Yi (2013) Bio-inspired data mining: Treating malware signatures as biosequences. [arXiv:1302.3668v1](https://arxiv.org/abs/1302.3668v1)
- Kim JS, Kluskens LD, de Vos WM, Huber R, van der Oost J (2004) Crystal structure of fervidolysin from *Fervidobacterium pennivorans*, a keratinolytic enzyme related to subtilisin. *J Mol Biol* 335:787–797. doi:[10.1016/j.jmb.2003.11.006](https://doi.org/10.1016/j.jmb.2003.11.006)
- Xu D, Yang Z (2013) Ab Initio structure prediction for *Escherichia coli*: towards genome-wide protein structure modeling and fold assignment. *Sci Rep*. doi:[10.1038/srep01895](https://doi.org/10.1038/srep01895)
- Sahi S, Tewatia P, Malik BK (2012) Modeling and simulation studies of human β 3 adrenergic receptor and its interactions with agonists. *Curr Comput Aided Drug Des* 8:283–295. doi:[10.2174/157340912803519633](https://doi.org/10.2174/157340912803519633)
- Karumuri S, Singh PK, Shukla P (2015) *In silico* analog design for terbinafine against *Trichophyton rubrum*: a preliminary study. *Indian J Microbiol* 55:333–340. doi:[10.1007/s12088-015-0524-x](https://doi.org/10.1007/s12088-015-0524-x)
- Fang Z, Zhang J, Liu B, Du G, Chen J (2016) Enhancement of the catalytic efficiency and thermostability of *Stenotrophomonas* sp. keratinase KerSMD by domain exchange with KerSMF. *Microb Biotechnol* 9:35–46. doi:[10.1111/1751-7915.12300](https://doi.org/10.1111/1751-7915.12300)
- Banerjee A, Sahoo DK, Thatoi H, Pati BR, Mondal KC, Sen A, Mohapatra PK (2014) Structural characterization and active site prediction of bacterial keratinase through molecular docking. *J Bioinform* 4:67–82