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Review Article

Striking Applications of Keratinase Enzyme Isolated from Various Natural Sources: A Review

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Abstract: Keratinase enzymes are protein hydrolyzing enzymes belonging to the serine hydrolase group and act specifically on keratin proteins to degrade them. Since keratin proteins are present in hair, hoofs, nails, beaks, animal skins, feathers and most of our green wastes, leaving them untreated may lead to environmental pollution. So, treating such material with keratinase enzyme in order to reduce environmental pollution is one of the striking applications of keratinase enzymes. Other applications may include dehairing, use in cosmetics, drugs, clothing and biofuel production. In this study, major focus has been given to applications of keratinases; however, the methods and sources of isolation of keratinase enzymes from different microbial sources has also been discussed. Bacteria as well as fungi possess the ability to produce extracellular keratinases which may be isolated and applied to several industrial sectors. Substitution of chemical agents with keratinase has been emphasized because in comparison to chemicals, keratinase enzymes are eco-friendly, biodegradable, do not produce harmful by-products and give very efficient results.

Keywords: Dehairing, Keratin, Keratinolytic enzyme, Serine, Domains, *Bacillus.*

1. INTRODUCTION

Keratins are the proteins which are usually present in two forms, namely hard keratins and soft keratins. Hard keratins mainly include the structural proteins which are prevalently present in finger nails, horns, beaks, upper layer of skin and mainly hair [1] (Fig. 1). Fibres of the keratin proteins are self-assembled into compact follicles that make up the structure of hair. The process of assembling up of keratin proteins into a complex hair is under the control of multiple genes, cytokines and growth factors [2]. In contrast to hard keratins, soft keratins are those which are abundantly present in tissues such as epithelial tissues.

Since the major focus of this study is on keratin proteins present in hair so we will discuss the classification of hair keratins here. The structure of wool keratin possesses great similarity with the hair keratin as shown in figure 2 [3]. Three types of hair keratin have been known yet [4]. First one is the alpha keratins, these ranges in size from 60 to 80 kDa. Being very less in sulfur content, these

Fig. 1. Sources of keratin. Different sources such as feathers, hair, nails, horns, hooves, and beak are shown. The hosts for these sources include human, bird, and animal. The hardness of these keratin materials is different in each case.

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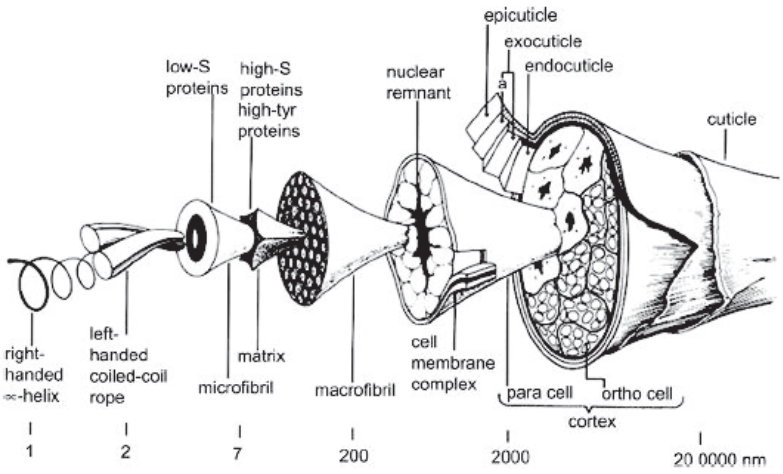

Fig. 2. The image above shows the structural scheme of keratin present in wool fibre which is closely related to human hair keratin with respect to its structure [3].

Family & Domains

Domains and Repeats

Feature key	Position(s)	Description	Actions	Graphical view	Length
Domain	19 – 77	Inhibitor_I9 InterPro annotation	Add BLAST		59
Domain	103 – 345	Peptidase S8 InterPro annotation	Add BLAST		243

Fig. 3. The image above shows the results obtained from (www.uniprot.org) that gives information about the domains present in keratinase enzyme.

comprise mainly of alpha helical domains. Overall, alpha keratins make up the structural class of proteins, as they reside in the fibre cortex of hair. Second ones are beta keratins (8 to 25 kDa in size), which are non-extractable, less studies class of keratins. These are usually present in the hair cuticle and perform protective functions. Third ones are gamma keratins, being very rich in sulfur content, these keratins are approximately 15 kDa in size [2,5]. Their size is comparatively smaller than other classes of keratin. These keratins help to maintain the cortical super structure by crosslinking the disulphide bonds in the hair [2]. All these types of keratins can be degraded by the enzyme keratinase which belongs to a class of protease enzymes.

Proteases, also called proteinases or peptidases are the ubiquitous and found in all form of life from prokaryotes to eukaryotes to viruses. They are essential enzymes for cell growth, and can hydrolysed peptide bonds (proteolysis). Proteases are the largest group of enzymes, which classified into seven broad groups (Serine proteases, Aspartic proteases, Threonine proteases, Metalloproteases, Cysteine proteases, Asparagine peptide lyases, and Glutamic proteases) that can accomplish the proteolysis by completely different catalytic mechanisms. Alternatively, proteases can be categorized into three groups due to their optimum pH in which enzymes are highly active such as alkaline (basic), acid and neutral proteases [6]. Proteases which account for 60% of the world's marketed enzymes, is responsible for many applications such as detergents, food and leather processing [3].

Keratinases (serine proteases) are one of the most effective and striking component of proteases as compared to others. Initially, they were classified as proteases of unknown mechanism but in 1990s due to their high sequence similarity with alkaline protease that have keratinolytic activity, and restraint by inhibitors of serine protease, therefore, they were defined as a serine proteases [7]. Keratinase is one of the venerable enzyme to hydrolyze the proteins rich in disulfide bond like hair and has caused no or little damage to leather. Therefore, keratinolytic protease is the most suitable tool for

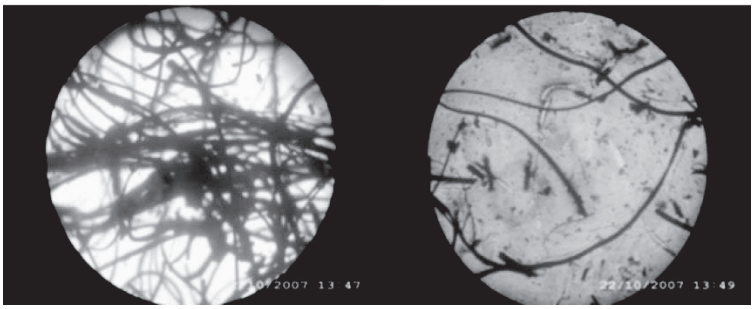

Fig. 4. This image shows how the structure of hair changes when it is treated with keratinase isolated from microbial sources [24]

various applications in industrial sectors [8].

1.1. Structure of keratinase enzyme

The enzyme keratinase (E.C. 3.4.99.11) is one of the serine hydrolases group that disrupt the disulphide hydrogen bonds in the keratin proteins [3,9]. According to uniport results, one of the protein keratinase produced by *Bacillus subtilis* contains two domains. First one is 59 amino acids long and encodes for inhibitor-I9; the other one is 243 amino acid long and encodes for peptidase S8. First domain occurs from 19 to 77 amino acid sequences and second domain occurs from 103 to 345 amino acid sequences (Fig. 3). The enzyme also has a metal ion binding site for calcium ion. This means that calcium ions act as the cofactors for keratinases; presence of calcium ions in the media can enhance the activity of keratinases. The structure of keratinase makes it very efficient in its function of degrading keratin proteins.

1.2. Why to employ keratinases?

Our daily green waste and animal waste includes plenty of keratins which remains undegraded due to their complexity. Such insoluble keratins may lead to environmental pollution if left untreated. So as a solution, such wastes are treated by keratinase enzymes which convert the waste into simpler as well as biodegradable substances [3]. One such example is shown in Fig. 4, that how the structure of hair changes when keratinase enzyme acts on it. The extracellular keratinases have been successfully isolated from several microbes by using several fermentation techniques and by optimising the conditions such as pH, temperature, and type of nitrogen and carbon source and the choice of microbe [9]. The keratinases from microbes are effective, biodegradable, economic, and provide much better results as compared to chemical treatments [10]. In this study, method of extraction of keratinase enzymes will be discussed and their industrial applications will also be considered.

2. DIFFERENT METHODS OF ISOLATION AND CULTIVATION OF KERATINASE ENZYME FROM NATURAL SOURCES

Work has been done since many years and keratinases have been isolated from different sources. Some of these methods for isolation and the sources from which keratinase have been isolated are discussed below.

2.1. Isolation from Waste Water Plant

A sample was collected from an activated sludge plant situated in a tannery and was taken to the laboratory for screening. For screening purpose, 2 g of agar was first dissolved in a solution of 10 mL fresh milk and 90 mL distilled water. After autoclaving, milk agar medium was poured into petri plates and inoculated with the sample taken from the activated sludge plant of the tannery. An incubation of 2 days at 30°C had shown the clear zone formation which indicated the presence of keratinolytic bacterium in the sample [3].

For growth and cultivation purpose, the medium of choice was feather meal medium whose contents were as follows in g/L: 0.5 NaCl, 0.5 NH_4Cl, 0.3 KH_2PO_4, 0.3 K_2HPO_4, 0.1 $MgCl_2.6H_2O$, 0.1 feather meal and 0.1 yeast extract with final pH of 7.0 to 7.5. The culture (5 mL) from overnight grown

pre-inoculum was added to 95 mL of this feather meal media and was incubated at 30°C at 150 rpm. Feather degradation was monitored by taking the OD of the media at 600 nm λ against distilled water as blank. Change in OD_{600nm} indicates that feathers being digested are a result of presence of extracellular keratinases acting on them [3].

2.2. Isolation from Bacteria

Keratinolytic bacteria were isolated using Nutrient agar medium having 1% (w/v) feather meal or skim milk. To check the presence of keratinophillic bacteria, soil (5g) diluted in distilled water (15 mL) by serial dilution followed by spread on agar medium and kept for 6 days incubation. Plates with clear zone around colonies will specify the presence of keratinolytic bacterial. After careful isolation, they were stored as glycerol stocks at -20°C [11,12].

A pre-isolated bacterial strains especially *Bacillus* spp. were used to cultivate the bacteria in fermentation media in order to produce extracellular keratinases. Basal salt media was used for the growth of this organism. The composition of this media in g/L was: 0.2 $CaCl_2$.H2O, 0.4 $MgSO_4.7H_2O$, 0.5 K_2HPO_4, 10 $NaNO_3$, 10 NaCl, 10 $NaCO_3$, 10 feather meal and 5 yeast extract. Pre-inoculum (3 mL) was taken and added to 97 mL of this media as 3% v/v inoculum. An incubation temperature of 45°C was given along with rotation of 180 rpm and the culture was incubated for two days following which the crude enzyme extract was obtained after centrifugation of the culture [13].

2.3. Isolation from Waste Dumps

A small soil sample was taken from waste dumps to the laboratory for scanning where it was diluted in distilled water and spread on to nutrient agar plates which were incubated at 37°C for 1 day. Once the colonies were observed, these colonies were taken and streaked on other agar plates which contained milk dissolved in it. Another incubation of one day was given at 37°C. Formation of colonies after incubation period indicates the caseinolytic activity of grown bacteria. For cultivation, 1% preinoculum was transferred to 100 mL nutrient broth having chicken feather meal as keratin source. The inoculated broth was incubated at 30°C in a shaking incubator at 150 rpm for 14 h. The resulting broth after incubation contained crude extracellular keratinase [14].

2.4. Isolation from Limestone Quarry

A limestone quarry was visited in India and some soil samples were taken from there. In the laboratory, these samples were dried and afterwards

Table 1. The table gives a brief account of species that have been reported to produce keratinase enzyme and the number of amino acids in each keratinase enzyme (source:https://www.ncbi.nlm.nih.gov/protein).

Sr. no.	Reported species	No. of amino acids	Molecular weight (kDa)	Accession number
1	*Bacillus subtilis*	362	37.22	AIY62812.1
2	*Bacillus sp. MKR1*	379	38.90	AEI59720.1
3	*Bacillus pumilus*	383	39.48	ACM47735.1
4	*Bacillus amyloliquefaciens*	382	39.15	AKR05134.1
5	*Stenotrophomonas maltophilia*	589	61.78	BAQ36632.1
6	*Geobacillus stearothermophilus*	546	59.68	AJD77429.1
7	*Bacillus velezensis*	382	39.15	AGC81872.1
8	*Bacillus thuringiensis*	347	36.94	APS24128.1
9	*Bacillus licheniformis*	379	38.89	AAY82467.1
10	*Pseudomonas aeruginosa*	301	33	6FZX_A
11	*Streptomyces albidoflavus*	360	36.4	AQX39246.1
12	*Actinomadura keratinilytica*	384	39.11	ASU91959.1
13	*Streptomyces sp. OWU 1633*	268	27.9	AAU94349.1
14	*Bacillus circulans*	383	39.48	AGN91700.1

crushed finely using a pestle and mortar to make a fine powdered sample. The solution (1%) of this sample was prepared by dissolving 1 g of sample in 99 mL distilled water and mixed properly. After giving incubation of 30 min at 25°C and 150 rpm, further serial dilutions were prepared from this sample. This solution was spread over the petri plates containing Horikoshi media. Successfully grown colonies were then selected for keratinolytic activity by streaking them on chicken feather media whose composition is as follows (w/v %): 0.5 KH_2PO_4, 1 feather, 0.5 Na_2CO_3 and 0.05 $MgSO_4$. Growth of colonies grown on this media had the keratinolytic activity [15].

For bacterial growth and cultivation of keratinase enzyme, chicken feather media was used in which feathers acted as keratin source and bacterial colonies grew producing extracellular keratinases. After inoculation of CFM with the sample, the broth was incubated at 30°C for 24 hours at 150 rpm. Feathers also acted as indicators as when they got degraded, production of keratinase was confirmed [15]. Work has been done on isolation of keratinase enzyme from several bacterial species. Characterisation of the isolated enzyme has been done and the number of amino acid per keratinase molecule has also been found out. Various bacterial species especially *Bacillus* spp. have the ability to produce keratinolytic enzymes (**Table 1**).

2.5. Isolation from Actinomycetes

Actinomycetes producing keratinases isolated from soil of poultry samples using serial dilution method. Soil sample (1 g) was suspended in distilled water (9 mL) and diluted up to 10^{-8}, prepared suspension was plated over AIA (with 5 mL L^{-1}glycerol) [16] and SCA of pH-7.3 [17] containing cycloheximide (50 µg mL^{-1}) as antifungal. The plates were incubated for 7–10 days at 28°C. By re-streaking, isolates were purified and screened. Pure culture of isolates were maintained ISP1 and stored in 20% glycerol stock at -20˚C for long term storage [1].

2.6. Isolation from Halophilic Microorganism

Keratinolytic halophilic microorganisms were isolated using serial dilution method. Halophilic medium for the isolation of bacteria containing peptone 0.2% (w/v), tri-sodium citrate 0.1% (w/v), yeast extract 1% (w/v), NaCl 10% (w/v), $MgSO_4$ 0.2% (w/v), agar 2% (w/v) with pH 7.5 was used. The medium with peptone 2% (w/v), glucose 6% (w/v), $MgSO_4$ 0.5%(w/v), yeast extract 1% (w/v), malt extract 1% (w/v), $FeSO_4.7H_2O$ 1% (w/v), NaCl 10% (w/v), K_2HPO_4 0.5% (w/v), and Agar 2% (w/v) was used for the isolation of fungi [18]. Bacterial plates were incubated for 24 h at 37°C and fungus plates were kept at 27°C for 6 days.

2.7. Isolation from Fungi

Mostly, research work has been done on several fungi which had been used as source to isolate keratinase enzyme. A brief account of ability of several fungi to produce keratinase enzyme has also been briefly mentioned in Table 2. The methods and sources of isolation discussed above are only few of all those studied yet. However, the prime focus of this study is enlightenment of significance of keratinase so we will discuss that how keratinases are used in several sectors.

Table 2. Different fungal species reported to produce keratinase enzyme (modified from Lange et al [46])

Sr. #	Genus	*No. of amino acids*	Substrate affinity	References
1	*Chrysosporium*	*C. keratinophilum, C. indicum*	Sewage sludge, hair	[47,48,49,50]
2	*Onygena*	*O. corvina, O. piligena, O. equina*	Feather, hair, bristle,hoof, horn	[47,51,52]
3	*Microsporum*	*M. canis, M. gypseum Microsporum*	Pig, sewage sludge, stratum corneum, nail, hair	[47,50,53,54,55]
4	*Arthroderma*	*A. gypseum, A. otae, A. benhamiae Arthroderma*	Sewage sludge, hair, horn, hoof	[47,50,56,57,58]
5	*Gymnoascoideus*	*G. petalosporus*	Hair	[48]
6	*Coccidiodides*	*C. immitis, C. posadasii*	-	[59]

7	*Aspergillus*	*A. fumigatus, A. oryzae, A. parasiticus, A. niger, A. flavus, A. terrus, A. sulphureus*	Poultry soil, feather, nail	[60,61,62,63,64,65,66]
8	*Trichophyton*	*T. rubrum, T. tonsurans, T. verrucosum, T. mentagrophytes, T. schoenleinii, T. vanbreuseghemii, T. terrestre, T. ajelloi*	Nail, skin, stratum corneum, sewage sludge, hair	[47,50,54,56,57,67-71]
9	*Paecilomyces*	*P. marquandii*	Nail and stratum corneum	[72]
10	*Talaromyces*	*T. trachyspermus*	Hair	[39]
11	*Scopulariopsis*	*S. brevicaulis*	Poultry farm and hair	[39,73]
12	*Doratomyces*	*D. microsporus*	Nail and stratum corneum	[72,74]
13	*Tritirachium*	*T. album*	Horn chips	[75]
14	*Myrothecium*	*M. verrucaria*	Feather	[76]
15	*Candida*	*C. albicans, C. tropicalis*	Feather	[57]
16	*Trichoderma*	*T. atrvoviride*	-	[77]
17	*Geotrichum*	*G. candidum*	Hair	[48]

3. STRIKING INDUSTRIAL APPLICATIONS OF KERATINASES

Keratinases can be solely utilized in the areas demanding harsh proteins degradation like nails, hair, feather, and also prions which can't be besieged by the conservative proteases [19]. Some of well-explored applications containing a theoretically huge market size have been particularized below.

3.1. Keratinous Wastes Recycling

The Keratinous wastes are chief by-product of the slaughterhouse, poultry, fur - and - leather processing industries, and are profusely spawned in numerous forms like hair, feather, hoof, horn, claws, nails, bristles, and wool [20]. The feather creates chief portion like poultry wastes along with 8.5 million tonnes round world and also the India subsidises 350 million tonnes over the year. Keratinous waste on the hydrolysis is transformed to the keratin hydrolysate that contains huge content of nitrogen, and is opulent in the hydrophobic amino acids. The characteristics of the keratin hydrolysate create it an extremely productive product along with the applications in various fields [21].

Numerous economically and effective methods are being developed merely for the keratin hydrolysates production. Unadventurously, keratin hydrolysates, particularly the feather meal have prepared only by the treatment along with an alkali, like KOH, NaOH, and $Ca(OH)_2$ at the great temperatures or the reduction along with the 2-mercaptoethanol in occurrence of the urea. The processing of the chemical may move to the degradation of heat-susceptible amino acids comprising methionine, tryptophan and lysine that may in produce non-nutritive amino acids, like lanthionine and lysinoalanine [22]. Because of the issues of the environment contiguous conservative chemical processes, the biotechnology through usage of the keratinolytic microbes as well as keratinases is promptly attaining ground. A miscellaneous group of the keratinolytic microbes have recognized that cultivate on or vitiate the keratinous wastes. It not only transforms waste to the productive hydrolysate, but also moves to concurrent manufacture of the keratinases.

Though, the processes have numerous restrictions like blockage because of unsolvable feather as well as the hair waste, unnecessary usage of the energy as well as lengthy incubation time merely for the degradation [10]. To compensate the restrictions, microbes-based process is being substituted merely by the enzymatic processes utilizing keratinases. Enzyme-based process is effective, reasonable, as well as time-saving.

The main limitation of utilizing the enzyme-

based process is manufacturing cost, though, which can be assuaged by the reusability of enzyme utilizing restrained measures. Nevertheless, widespread research requires to be completed on the reusability of the keratinases. Certain researchers have recognized the manufacture of feather meal utilizing keratinase restrained on the nanoparticles and by means of bio-restrained keratinases [15].

3.2. Medical Applications

The Keratinases are capable to invade skin as well as nail keratin, and therefore, discover application as preservative to enhance the effectiveness of the up-to-date drugs. They are being employed for numerous conditions of the skin for instance corn, callus and acne [19].

3.2.1. Keratinases used in trans-ungual delivery of drugs

Nail syndromes range from the comparatively inoffensive conditions such as pigmentation, to the debilitating as well as painful states at which the nail unit can be, hypertrophied, dystrophied, infected and inflamed. The most communal infection of fungi of nail, is onychomycosis, has a great pervasiveness rate along with roughly 700 million people anguishing from this situation worldwide. Maximum diseases of the nail are problematic to treat specifically ones having contaminations happen beneath nail plate. They necessitate a prolonged treatment period as well as face foremost issue of non-invasiveness, reoccurrence, and drug specificity, removal of the side impacts as well as enhanced compliance of patient [23].

Inopportunely, many of up-to-date medicines of the nail have restricted effectiveness because of pitiable permeability of drug through nail plate. Presently, numerous physical, chemical as well as mechanical methods are utilized for the trans-ungual distribution of current medications [24]. The mechanical methods, like nail avulsion as well as nail abrasion, are painful as well as invasive.

The physical methods involve hydration, carbon dioxide laser, occlusion as well as etching. The chemical methods comprise the usage of the keratolytic agents like thioglycolic acid, urea, salicylic acid and papain in amalgamation with the oxidizing agent like hydrogen peroxide. Chemicals like thioglycolic acid, N-2 mercaptoethanol, N-acetyl cysteine, mercaptoethanol, as well as N-acetyl cysteine are utilized at most of the times for degrading the surface of the nail as well as enhancing the permeation of nail plate. The compounds contain a powerful odour, which are acidic in nature, and comprise a prospective to respond with particular drug combinations [7].

The disadvantages of prevailing methods can be efficiently concerned by the usage of keratinases. Keratinases as the molecular scissors are splitting the hard keratin protein that establishes chief portion of the nail plate, thus slackening plate as well as improving trans-ungual permeability of the drug [25]. Keratinases occur on mutually intercellular matrix which connects cells of nail plate together and also dorsal nail corneocytes merely by disintegrating surface. Keratinase merely from the *Paecilomyces marquandii* was exposed to incompletely disturb nail plates as well as enhance the permeability of the drug. A multifaceted subtilisin-γ-glutamyl transpeptidase, for instance, keratinase KerN has been explained to increase delivery of the drug via nails. Moreover, an insufficient keratinase-based marketable preparation involving FixaFungusTM, Pure100 Keratinase as well as Kernail-Soft PB, are obtainable in market only for considering nail disorders [26].

Besides infections of nail, the keratinases can be used merely for the permeabilization of the skin tissue to improve the delivery of the drug over surfaces of the skin. The keratinolytic agents eliminate the hyperkeratotic scratches, refining the introduction of inflamed surface of the skin to the current drugs [27], established skin agent merely by restraining keratinase to the porous sheet that releases skin as well as recovers the permeability of the drug. To feat the probable of the keratinases like well-organized ungual enhancers as well as the permeabilizers of the skin tissue, widespread research wants to be assumed. Keratinases can develop an important preservative of prevailing nail lacquers. Absence of appropriate in vitro approaches to measure the degree of permeation of the drug is primary exertion. More premeditated approaches accompanied by human as well as animal trials want to be supported out for the commercialization of the keratinase-created drug delivery preparations [22].

3.2.2. Calluses and Corns Removal

Calluses as well as Corn, also known as hyperkeratosis, are excruciating thickenings of the weakened skin which frequently form the dorsal surface of fingers as well as toes. During treatment, podiatrists recommend the usage of keratinolytic agent like salicylic acid. It softens keratin which creates the chief portion of the corn as well as dense layer of the weakened and dead skin [28]. The usage of the keratinases is natural as well as greener substitute to usage of the salicylic acid. Numerous groups related to research have discovered usefulness of the keratinases merely for eradicating horny skin layer.

3.2.3. Treatment of acne

Acne is a mutual skin problem which happens because of hindering of sebaceous gland by occurrence of unwarranted keratin. Like keratinases can liquefy departed cells as well as the keratin which blocks sebaceous glands, this can be functional to the acne treatment. A keratinase-related product which can be effective adjuvant in the acne therapy has been untested since 2001 [10].

3.2.4. Bio-safety to prions that are infectious

One of the greatest authoritative applications which have revolutionized the keratinase research is in field of the decontamination of the prion. Prions are the transferable agents which cause lethal as well as communicable brain diseases [5]. These transferable biomaterials are evolving contaminants related to the environment that are emitted into environment over many routes for instance disposal of the mortalities, body fluids, PrPSc polluted effluents from the hospital, slaughterhouse as well as research facilities. It amasses via ingesting of the meat as well as reprocessed the waste products for instance bone meal of infested animals. Straight passageway of PrPSc from environment towards host happens by inhalation or ingestion while secondary passage happens via medical devices specifically the stereotactic electrodes. Disposal as well as storage of the biological and clinical wastes is the stuff of worldwide concern which must be distributed with immediately [26].

Numerous physical and chemical approaches of sterilization and disinfection safeguarding the decontamination of prion have been deliberate widely by Weber et al [29]. Though, the prion disinfecting methods are not only eco-unfriendly, punitive, and also the energy intensive, but also do not guarantee to comprehensive damage of contamination. Moreover, their constant application can harm the medical related devices, thus, warning their usage in the hospitals. To cope with degradation of infectious the domain of the prion proteins, enzyme-related practices could be an innocuous resolution [30].

Keratinases are talented candidates for the decontamination of the prion like they break β-keratin with improved rate than the conservative proteases, on account of structural similarity of extremely accumulated β-pleated structure of the prion protein (PrPSc) of feather, abundant in the β-keratin. Cheng and co-workers [31] were the first one to determine the dilapidation of the PrPSc infested brain tissues by the KerA of *B. licheniformis* PWD-1. Numerous keratinases from assorted group of actinomycetes as well as the bacteria have been exposed to damage prion proteins in the lab experiments. Keratinases from the *Nocardiopsis* sp. and *Streptomyces* sp. TOA-1 have been recognised to destroy the amyloid prion proteins nevertheless under dangerous situations for instance alkaline pH, as well as high temperatures. Additionally, limited keratinases from the thermophilic organisms for instance genus *Thermosipho*, *Thermococcus* and *Thermoanaerobacter* have been damaged PrPSc deprived of pre-treatments, and also have been working for emerging prion free as well as non-pathogenic animal meal. Moreover, a collection of serine proteases generate from the lichens *Parmelia sulcata*, *Lobaria pulmonaria,* as well as *Cladonia rangiferina*, and also keratinase from *B. licheniformis* have exposed the prospective to damage transferable prion proteins underneath slight conditions. Recently, a profitable product specifically Pure100 Keratinase propelled by the Proteos Biotech has been engaged to disinfect surgical instruments which are vulnerable to contamination of the prion [32].

Nevertheless, many enzymatic approaches want to be functional in amalgamation with the other treatment regimens such as alkali, detergents, or high temperatures. The regimes are normally dangerous and environmentally unfavourable. Therefore, search endures for recognizing catalytically additional detailed enzymes which can

abolish infectivity of the prions during reasonable conditions deprived of denaturing pre-treatment stages [5].

3.3. Keratinases as Efficient Substitutes Compared to Commercial Proteases

Keratinases contain conservative proteases along with their probable to damage both insoluble as well as soluble proteinaceous substrates, and also may demonstrate to be improved than predictable proteases in the protease-prevailing sectors.

3.3.1. Keratinases as feed additives

The animal feed, frequently made of vegetable as well as cereals proteins together with the products of meat are problematic to assimilate as well as digest by the animals. The utilization of feed can frequently be enhanced by accumulation of the enzymes to feed. A great array of the feed enzymes for instance lipase, xylanase, cellulase, α- amylase, β-glucanase, pectinase, protease, and phytase are in market like additives of the feed in the diets for poultry, fishes, cattle, sheep and pigs. The usage of the proteases is recognized for the quicker development as well as performance of the young ones by enhancing nutritional value as well as digestibility of prevailing dietary proteins.

Keratinases contain originate their location in the feed enzymes that keratinases can cleave the PrpSc proteins, and also create the products of the meat innocuous for the ingestion by the animals. The Keratinolytic proteases have been fine recognized to provide improved consequences by accompanying meat as well as cereal-related diets. They had affirmative impacts on performance of the growth to the young ones, enriched the utilization of the amino acid as well as the structure of the gut villus. Moreover, enhanced the consumption of the keratinase accompanied diets has moved to lessening in the requirement of the feed [33].

Keratinase derived from the *B. licheniformis* PWD-1 advanced in the trade name Versazyme is utilized like the nutraceutical product and also has move to momentous enhancements in the performance of the broiler. The accumulation of the Versazyme in pelleted as well as mashed diets exposed valuable impact on initial growth and feed utilization of broilers. Additional keratinase of the *B. licheniformis* PWD-1 promoted under the product name Cibenza DP100TM is utilized for maintainable as well as fruitful growth of the piglets [31, 34].

3.3.2. Detergents

The proteases have been extended to industry of the detergent merely for supporting in the proteinaceous stains removal. Nevertheless, keratinases are supposed to require improved detergency like they are wide-range proteases along with restored substrate specificity merely for both the insoluble as well as soluble substrates of the protein. They can simply hydrolyze immobile proteins upon surface, and it can also eradicate stains involving keratinous soiled blood stains, cuffs as well as collar. Additional forthcoming application of the keratinases is in field of the wiping up the drain pipes as well as outlets stopped with the hair as well as additional keratinous matters. Itsune et al [35] have established scrubbing agent conformation organized by the compounding keratinase as well as the non-sulfur reducing agent which is able to wiping smarmy matter committed on drain outlet of bathroom and the dirt triggered by the pollutants for instance hair as well as scales, along with huge effectiveness in innocuous manner. The profitable product such as BioGuard Plus is accessible which integrates a mixture of diverse enzymes involving keratinases merely for wiping out drain pipes as well as tanks [2].

3.3.3. Leather dehairing

The processing of the Leather comprises four chief stages viz. dehairing, soaking, tanning as well as bating. Conservatively, the soaking is achieved utilizing the alkali, the dehairing is the sulfide-related, although tanning as well as bating includes the usage of solvents, lime and salt. The usage of the punitive chemicals creates the industry of the leather exceedingly polluting which subsidises to foremost disposal problems of the effluents [14].

The usage of the enzymes is greener substitute moving to lessening in the pollution in the environment and also enhanced quality of the leather. Many profitable proteases for instance Pyrase, NUE, Clarizyme, as well as Aquaderm are accessible that are utilized in dehairing, bating as well as soaking. Nevertheless, the comprehensive replacement of the chemical processes merely by enzymes is not informal because of economics

of enzyme-related processes. This matter can be concerned by usage of the proteases which have improved speciality to the hair, and also are more effective catalytically and will be obligatory in slighter amount [36].

Moreover, the keratinases wanting the collagenolytic, and containing slight elastolytic happenings are progressively being discovered due to the dehairing process. The enzymes would assist in discerning interruption of the keratin tissue in follicle, thus, dragging out complete hair deprived of distressing tensile strength of the leather. Like for industry of the leather, the keratinases derived from the *Aspergillus nidulans*, *Bacillus subtilis* S14, *B. subtilis* KD-N2, *Bacillus sp*. PPKS-2, *Paenibacillus woosongensis* TKB2, *Trichoderma harzianum* MH-20 displays extraordinary dehairing competences deprived of the collagen deprivation. An enzyme-related dehairing entirely eradicates the want merely for the toxic sodium sulfide in the leather processing. This environment-friendly dehairing method contains most significant compensations, primarily on the ultimate quality of the leather product, and overwhelming pollution problems in environment produced by conservational chemical processes, and also products of the waste [21].

3.3.4. Textiles

The wool is mechanical protein fibre considered by great degree of the cross-related disulfide bridges (S–S) which discuss the mechanical resistance as well as strength to the deprivation by the proteases. It is accredited to overlying layers of the cuticle categorized by exo-, epi-, and also endocuticle wrapping the external surface of fibers. Epicuticle is abundant in the lipids although endocuticle as well as exo- are encompassed of the keratin, abundant in the disulfide bonds [23].

The structure creating cuticle plays significant part in felting contraction of fibers in washing and disturbs the dyeing. Another the Chlorine-Hercosett process, including the usage of absorbable organic chlorides (AOX), has been engaged in processing of the wool over the last 30 years to switch the felting contraction of the wool fibers. Nevertheless, this treatment moves to weight damage of fibers with the clearance of dangerous chemicals in environment. The other chemical related important concluding progressions, comprising scrubbing (cleaning), dyeing or bleaching, cause expulsion of a dissimilarity of poisonous chemicals. Moreover, the procedures are not only time-consuming as well as energy intensive, but also have a propensity to damage the simple material. The usage of the enzymes is pragmatic as an eco-friendly safe substitute as well as numerous protease-related commercial products for instance Savinase, Esperase, Alcalase, as well as PeriZyme Tuggumm Type EX have been discovered in diverse textile concluding progressions over the last decade. Nevertheless, in accumulation to eliminating cuticle, proteases infiltrate deep in wool fiber thus destructing it and moving to damage of tensile strength as well as the weight of the fibre [7]. This issue has been assuaged by enhancing molecular weight of the proteases via alterations of chemicals using polymers for instance glutaraldehyde, Eudragit S100 or PEG. Utilizing keratinases that would selectively mark scaly keratinous layer of wool deprived of harmful other portions of fiber may be anticipated substitute. Numerous keratinases derived from the *B. licheniformis* L11, *B. thuringiensis*, *Pseudomonas sp*., *Bacillus cereus*, *Fusarium* sp., and *Stenotrophomonas maltophilia* DHHJ have been familiar to increase stroked-shrink hostility dyeing deprived of harm of fiber weight. The action of the keratinases has been enhanced additional by coalescing them along with lipase or cutinase. The strength fibre has been enhanced by employing transglutaminase [37].

Therefore, keratinases unaided or in amalgamation with the other enzymes can help in emerging necessary formulations for better-quality wool processing. The raw silk wants degumming to eliminate fibrous protein, sericin which strengthens the fibroin fibers self-possessed, thus as to afford the soft feel as well as fibers luster. This method is significant for following dyeing. Conservative degumming methods are treated along with the soap, oxidizing agents and alkali at greater temperature in agitation. These situations change the chemical as well as physical properties of the fibers moving to dilapidation of main material of the silk. Enzymatic behaviours of proteases for instances Papain Degummase, Pepsin Trysin, Savinase Alcalase, Protease N Amano, Protease A Amano, Palkobate and Protease M Amano are in emphasis over the conservative method. Though, many proteases are considered by less degree of

specificity to the sericin. Therefore, enzymes along with improved specificity are necessity of hour as well as keratinases along with their extensive substrate range may verify to be practicable substitutes [38].

3.4. Prospective applications of keratinases

Along with the well-recognized application fields, keratinases comprise impending to be utilized in most of the areas providing selective deprivation of rough proteins such as hair as well as skin. Researchers have begun discovering some innovative applications like hair as well as cosmetic preparations, the elimination of the earwax, and also pearl bleaching.

3.4.1. Earwax removal

Earwax that is also referred to the therapeutic term cerumen, is hydrophobic defensive covering in ear canal of the humans and also other mammals. Earwax principally comprises of the shanty layers of the skin, along with 60 % of the earwax comprising of keratin. Cerumen accumulation is reacted by the softeners comprising cerumenolytic agents involving sodium bicarbonate, glycerine, arachis oil, carbamide peroxide, dichlorobenzene, triethanolamine, turpentine, hydrogen peroxide, and urea. Nigam [39] has revealed configuration for eliminating the human cerumen which comprises bicarbonate in aggregation with amalgamation of the enzymes involving protease/ keratinase, amylase and also lipase. They have discovered the opportunity of utilizing enzymes like Trypsin, Pancreatin, Collagenase, Subtilisin, Carboxypeptidase, Keratinase, Papain, Bromelain, Elastase and Aminopeptidase. The enzyme-related cerumenolytic configurations are commercially safe feasible and efficient in removal of the cerumen from external ear canal. The huge conc. of the keratin in the cerumen creates keratinase/ keratinolytic protease a very possible candidate to be discovered additional for claim [19].

3.4.2. Pearl bleaching

Pearls are designed like a defence appliance in contradiction of possibly intimidating irritants in the living shelled molluscs like an oyster. The organic constituent known as “Mother of pearl” or “Nacre” is concealed over interfering irritant. Nacre is made chiefly of the crystallized calcium carbonate and also conchiolin, black coloured organic protein. In pearl establishment, the organic impurities like able cells of the mother of the pearl oysters, mucilage as well as necrotic portion of the mantle tissue pieces are surrounded in pearls and they want to be handled to improve their quality of the gem.

The Pearls are exposed to bleaching behaviour which assists in blanching them, evening out the irregularities of the color and overwhelming brown color of the conchiolin. The methods of bleach utilized are very slight like hydrogen peroxide; permitting mild lightening of pearl nacre deprived of detrimental the quality since surface of the pearl is polluted with organic impurities such as mucilage, tissues, as well as cells. Zhang et al [40] have discovered the usage of keratinases in bleaching of the pearl. Keratinases can be utilized in preliminary treatment to eliminate keratin impurities on pearl surface monitored by conservative bleaching or processing methods [28].

3.4.3. Cosmetics/personal care products

Keratinases have been engaged in formulation of the cosmetics for hair as well as skin. For the skin, keratinases have been further in configurations for whitening of the skin, dispelling of the freckle, and also bleaching. Keratinases can be utilized for the removal as well as exfoliation of the stratum corneum. Keratinases have been further to compositions hair for instance conditioner, hair gel, and shampoo where they perform a double role in refining color of the hair, luster and quality with concurrent cleaning as well as elimination of layers followed on hair [13].

3.4.4. Processing of edible bird’s nest

Nests constructed by an insufficient species of the swiftlets are spent by the humans internationally, like great-value fragility or like medicinal Applied Microbiology Biotechnology food. Nests are comprised of gelatinous constituents, and comprise intertwined feather and also fluff like impurities. They are cleaned by the processes for instance hand picking, sieving as well as hot water treatment which are time-consuming and also unproductive. The huge cost or demand for the nests has moved their constructers to approve some dangerous practices like reacts with the silicates as well as peroxides. Plumages as well as feather are chief impurities, formulations related to specialty

enzymes like keratinases, which can selectively spasm insoluble proteins may demonstrate to be an efficient method of the processing the nests. Nevertheless, widespread research wants to be lead to authenticate this, and also an enzyme has to obtain GRAS status to be oppressed for the application of the food [4].

3.5. Other applications

The application areas emphasized above practice the chief contribution of protease market. Moreover, of these, proteases are utilized to smaller extent in fields like the synthesis of peptide, elimination of the silver from the photographic films and also contact lens cleaning. More intensive research wants to be completed to damage the intrinsic advantages of the keratinases over the proteases in sectors.

4. DISCUSSION AND LATEST TRENDS

In recent time, keratinase is the most noteworthy member of proteases group of enzymes that can effectively hydrolyse the tough insoluble protein and polypeptide molecules into amino acids [11,41]. Keratinase are ubiquitous in nature but as compared to fungal, animals and plants, bacteria are the attractive source of keratinolytic enzymes as they can be cultivated easily in lab and produced industrially important enzyme in large amount in a short time by optimized fermentation methods. Keratinolytic enzymes are produced by organisms only in the presence of keratin substrate. Bacterial protein has long shelf life without reduction in activity and can be stored easily [42]. Naturally, bacterial keratinases are extracellular and soluble protein. They are secreted directly into the fermentation culture broth, so the downstream processing of these extracellular keratinolytic enzymes are easy as compared to the enzyme obtained from other sources [41].

Keratinase have great sequence homology with alkaline proteases and these are catalytically active in neutral to alkaline pH range and nearly thermophilic temperature, which is the most prominent feature of alkaline proteases. Therefore, keratinolytic enzymes are define as serine proteases which belong to alkaline proteases (EC.3.4.21-24, 99), which are either have a metallo-type or serine centre. They are widely used in food, detergent, leather, and pharmaceutical industries [42,43]. So far, the production of keratinase from poultry soil bacteria has been discussed and applications of the enzyme have also been illuminated.

Despite being successfully applicable in many emerging fields, scientists are still finding new ways to make keratinase more efficiently applicable. One such example is isolation of keratinase from *Meiothermus taiwanensis* WR-220 which is a thermophilic bacterium and shows maximum keratinase activity at temperature around 65°C [44] providing an eco-friendly way to convert keratin wastes to valuable amino acids. Since keratin is very difficult to degrade and using enzyme hydrolysis method assisted with high temperature can provide very efficient keratinolytic activity. Moreover, the gene for keratinase enzyme from *Meiothermus taiwanensis* WR-220 was ligated into an expression vector and further transformed to a bacterium to further enhance its expression [44].

In another research, a chicken feather-degrading bacterium *Fervidobacterium islandicum* AW-1 was isolated from a hot spring of Indonesia. The optimum temperature for the growth of this bacteria was 70°C and it could degrade chicken feathers. The crude sample of extracellular enzyme extract obtained by the fermentation of this bacteria hydrolysed keratin efficiently at 90°C [28] This reveals that using high temperatures for keratin degradation can provide best results when compared with the results of degradation at lower temperatures i.e. 35 to 40°C by mesophilic bacterial keratinase. Such thermostable keratinases can provide quick and efficient dehairing in leather industry.

Experiments are also done using keratinolytic fungi including *Fusarium* sp. strain 1A, *Trichophyton* sp., *Cladosporium* sp., *Chrysosporium* sp, *Microsporum* sp., *Trichoderma* sp., and *Phytophthora* sp. isolated from the soils where there were keratin deposits [32]. Horse hair were provided as source of keratin which is a substrate for the keratinase enzyme. The structure of hair was observed over a period of several days under SEM to check the effect of keratinase and later it was revealed that *Fusarium* sp. strain 1A, *Microsporum sp. and Chrysosporium* sp. have the greatest ability for degradation of keratin [32].

The growing interest in thermophilic microorganisms and their potential biotechnological applications explains the increasing number of studies in extremophilic microorganisms around the world [45]. Thermophilic keratinases are being isolated from different regions of the world and are being employed for several purposes. The only reason is that when we need to degrade keratin, a higher temperature helps to weaken the bonds within a keratin molecule and afterwards enzymatic hydrolysis becomes much more efficient and quick. So, the use of thermophilic bacteria for isolation of keratinase rather than mesophilic bacteria have become the choice of the scientists as per the latest researches [45].

5. CONCLUSION

With growing emphasis on the eco-friendly atmosphere, and use of biocatalysts in industrial processes gained significant attention in this era. There is a great need to search new industrially relevant enzymes from microorganisms that have abilities to accomplish demand of industries. Keratinases are the useful industrial enzymes, we have so far discussed most of the applications of keratinases and their major roles in upcoming biotechnological era. The use of keratinases in previously mentioned fields can solve many problems such as reuse wool waste, poultry feather waste degradation, used as bio-control (keratinases use against plant pathogens because of having antagonistic activities), in detergents, has bio-fertilizing potential and have various agricultural applications. The usage of bacterial keratinolytic enzymes to improve the production of agricultural crop has developed as an alternative and sustainable tool to meet challenges. In these days, scientists have focused to use organic (composted) wastes as fertilizers that will decrease the prices of chemical fertilizers (commercial fertilizers) and it will be more favourable for our ecosystem. Hence, keratinase is a productive and economic choice for industrial and biotechnological applications.

6. CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests. We assure the quality and integrity of our work. This study is completely independent and impartial; all points taken from other authors are well cited in the text.

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