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# Keratinases: emerging trends in production and applications as novel multifunctional biocatalysts

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## Abstract

Keratinases are proteolytic enzymes capable of degrading rigid and insoluble keratinous proteins present in skin and appendages. They are produced in the keratinous substrates such as feather, hair, wool, nail, horn and hoof by microorganisms. They are mostly serine proteases, although there are very few reports about metallokeratinases. Keratinases are active over wide range of conditions, and are useful in biorecycling of keratin wastes into feed and fertilizers. They also have potential applications in leather, cosmetic, textile, biomedical and detergent industries. The promising applications of keratinases also extend to energy generation and green synthesis of nanoparticles. Owing to their ubiquitous biotechnological applications, techniques such as immobilization, optimization strategies, protein engineering and DNA recombinant technology have been used to improve their activities and stabilities thereby widening the scope for commercialization. This review chronicles recent trends in the production and multi-functional applications of keratinases.

**Keywords:** Biocatalysts; bio-products; keratinases; keratins; proteases.

## 1. Introduction

Keratin-rich materials are generated abundantly as wastes from meat and poultry processing industries. They are recalcitrant to hydrolysis by common proteolytic trypsin, pepsin, and papain, thereby constituting nuisance to the environment. The mechanical stability of keratin and its resistance to microbial degradation are due to the tight packing of the protein chain, either in α-helix (hair α-keratin) or β-sheet (feather β-keratin) structures, and their linkage by cystine bridges that have a high degree of cross-linkages by disulfide bonds, hydrogen bonding, and hydrophobic interactions (Gupta & Ramnani, 2006; Mazotto *et al*., 2011).

Feather produced in large quantities as by-products of poultry processing industries are the largest reservoirs of keratin. Traditional methods for the treatment of feather wastes, such as burning, land filling, steam pressure cooking and strong alkali or acid hydrolysis not only cause environmental problems, but also require enormous energy for processing, thereby resulting in destruction of some essential amino acids. Therefore, development of ecofriendly method for the degradation of feather that will guarantee production of useful products becomes imperative. In this connection, degradation of feather by keratinolytic microorganisms represents an alternative method (Jayalakshmi *et al*., 2012).

Keratinases are serine or metalloproteases that can degrade fibrous and insoluble keratinous materials. They are robust enzymes with wide range of temperature and pH values with optimal activities at neutral to alkaline at temperatures ranging from 40-70°C. Keratinases are also active over wide range of substrates including keratins, haemoglobin, fibrin, gelatin, and casein. However, there is no uniformity in the determination of their activities due to diversity in the structure of keratin substrates used for such determination, and the definitions of keratinase activity (Gupta & Ramnani, 2006; Lateef *et al*., 2010). Their mode of action involves complex systems of sulfitolysis and proteolysis (Gupta & Ramnani, 2006). Characteristics of some keratinases are shown in Table 1.

Keratinase is a promising tool in various biotechnological applications such as bioconversion of keratin wastes to animal feed and nitrogenous fertilizer, cosmetic and detergent industries, medical

and pharmaceutical industries, textile manufacturing and leather industries. They are also applicable in the degradation of prion proteins, as pesticides, and in the production of nanoparticles, biofuel, biodegradable films, glues and foils (Gupta *et al*., 2013a; Revathi *et al*., 2013). Investigations into the microbial production of keratinases have been intense in the last decade, partly due to their ubiquitous production, and various potential applications in diverse areas of human endeavours. The intense research in this area of enzymology is evident in the number of research articles that appeared in reputable journals in the last decade. This review attempts to discuss current trends in the microbial production of keratinases and their versatility as novel biocatalysts with increasing multi-functionality.

## 2. Production of keratinases

### 2.1. Keratinase production in submerged fermentation

Microbial keratinases are predominantly extracellular, when grown on keratinous substrates and intracellular keratinases have also been reported (Onifade *et al*., 1998). In most cases, keratin serves as the inducer. However, non-keratin substrates such as soy meal are known to induce enzyme production. Two steps have been assumed to be involved in keratinolysis: sulfitolysis i.e the reduction of disulfide bonds and proteolysis (Gupta & Ramnani, 2006). Majority of reports on keratinase production are under submerged shaking/static conditions (Lateef *et al*., 2010; Cai *et al*., 2011). It is difficult to compare the production condition for keratinase due to variety of organisms and the methods of cultivation. Furthermore, carbohydrates such as glucose (simple sugar) have been reported to suppress the synthesis of keratinase due to catabolite repression (Daroit *et al*., 2011), while complex sugars like starch have shown to enhance the synthesis of keratinases (Syed *et al*., 2009).

### 2.2. Keratinase production in solid state fermentation

Substrates such as feathers, hair, horn and sugarcane bagasse have been used as inducers for the microbial production of keratinases under solid state fermentation (Al-Musallam *et al*., 2001; Al-Sane *et al*., 2002; Al-Zarban *et al*., 2002; Awad *et al*., 2011; Paul *et al*., 2013a). Similarly, it has been shown that addition of 0.1% soybean flour to feather medium increased keratinase production by *Bacillus* sp. PPKS-2 under solid state fermentation (Prakash *et al*., 2010a). In a study conducted by El-Gendy (2010), an endophytic keratinolytic strain of *Penicillium* sp was reported to have significantly produced keratinase under solid state fermentation, using different agricultural and poultry wastes.

### 2.3. Optimization of culture conditions for keratinase production

Different optimization strategies have been reported to enhance the microbial production of keratinolytic enzymes. These strategies include Plackett-Burman design, Box-Behnken design, central composite design, one-variable-at-a-time (OVAT), response surface methodology and statistical design. The Plackett-Burman factorial and Box-Behnken designs used by Liang *et al*. (2010) increased the keratinase production by thermophilic *Myceliophthora thermophila* strain by 6.4-fold. Keratinolytic activity of two recombinant strains of *Bacillus cereus* was increased in a 1% chicken feather containing mineral medium optimized with the addition of yeast extract and corn oil (Ouled-Haddar *et al*., 2010).

According to Harde *et al*. (2011), the use of one-factor-at-a-time strategy, an L8 orthogonal array design and response surface methodology improved the keratinase production by *Bacillus subtilis* NCIM 2724. Also, in the study conducted by Awad *et al*. (2011), a Box-Behnken design was reported to improve the production of keratinase in solid-state fermentation by *Bacillus pumilus* GHD using sugar cane bagasse. The production of alkaline β-keratinase by a strain of *Brevibacillus* sp. after 48 h of incubation was optimized by response surface strategy (Rai & Mukherjee, 2011). However, three strategies of temperature-shift procedure, two-stage DO control and fed-batch process in a fermenter induced a 62.2 % improvement of keratinase yield from wool degradation by a strain of *Stenotrophomonas maltophilia* (Fang *et al*., 2013). Therefore, optimization designs have proved to be useful and powerful tools for the development of optimal medium compositions and culture conditions, for the production of keratinolytic enzyme.

### 2.4. Mutagenesis

Manipulations of keratinolytic organisms by physical and chemical mutation tend to improve the production and property of keratinases. An induced mutant of *Bacillus subtilis* using N-methyl-N’-nitro-N-nitrosoguanidine has been reported to produce keratinolytic activity of about 2.5 times that of wild-type strain (Cai *et al*., 2008). A combined effect of mutagenesis by gamma radiation and ethyl methansulfonate (EMS) improved the production

of alkaline protease by *Penicillium chrysogenum* NNRL 792 strain (Afifi *et al*., 2013) successively from 40.0 U/g for wild strain, to 62.92 and 120 U/g for gamma mutant G9 and EMS-1 mutant respectively. The purified mutant alkaline protease from EMS-1 was reported to have higher thermo- and pH stabilities.

Protein engineering technologies such as directed evolution, site-directed mutagenesis, truncation, and terminal fusion have been developed to improve the industrial use of natural enzymes as biocatalysts (Yang *et al*., 2014). Site-directed mutagenesis was recently reported to have improved the keratinolytic activity of microbial keratinases (Liu *et al*., 2013b). Liu *et al*. (2013b) demonstrated the use of computer algorithm to predict folding free energy (ΔΔG) as a result of amino acid substitution in the keratinase of *B. licheniformis* BBE11-1. Use of the algorithm in combination with molecular modification of homologous subtilisin allowed the introduction of four amino acid substitutions (N122Y, N217S, A193P, N160C) into the enzyme by site-directed mutagenesis. The introduction of the four amino acid substitutes and the subsequent expression of the mutant genes in *B. subtilis* WB600 led to the production of an enzyme with both 5.6 and 8.6 fold improvements in catalytic efficiency and $t_{½}$ value at 60 °C, respectively.

### 2.5. Cloning and expression of keratinase gene

Increase in the keratinase yield could also be achieved using DNA recombinant technology. Isolation and cloning of keratinase genes are important to enhance the enzyme yields to allow for commercialization. Also, the sequencing of keratinase gene serves as a prelude to phylogenetic analysis of the enzyme, which can be useful in deciphering structure-function relationships (Gupta & Ramnani, 2006). The keratinase gene from *Bacillus licheniformis* MKU3 was cloned and successfully expressed in *Bacillus megaterium* MS941 and *Pichia pastoris* X33. The recombinant *B. megaterium* and *P. pastoris* strains produced 3-fold and 2.9-fold increases in keratinase yield than their respective parent strains (Radha & Gunasekaran, 2009). Two strains of feather-degrading *Bacillus cereus* designated as 23/1 and 6/2 were transformed with alkaline proteinase gene using a plasmid vector p5.2. The keratinolytic activities of the two strains in feather medium increased approximately by 3.5 and 4.15-fold, respectively (Ouled-Haddar *et al*., 2010). Similarly, the *ker* gene encoding keratinase was isolated from *Bacillus licheniformis* BBE11-1, cloned and expressed in *Bacillus subtilis* WB600 under the strong $P_{HpaII}$ promoter of the pMA0911 vector. The recombinant keratinase exhibited very remarkable properties (Liu *et al*., 2013a). Most recently, Fang *et al*. (2014) reported the isolation of two keratinolytic genes (KerSMD and KerSMF) of *Stenotrophomonas maltophilia* BBE11-1 with a modified TAIL-PCR (thermal asymmetric interlaced PCR) method based on the N-terminal amino acid sequences of mature keratinases. These two keratinase genes encode serine proteases with PPC (bacterial pre-peptidase C-terminal) domain, were successfully expressed with the help of *pelB* leader in *E. coli* cells. It was observed that recombinant KerSMD (48 kDa) showed a better activity in feather degradation, higher thermostability and substrate specificity than KerSMF (40 kDa). It had a $t_{1/2}$ of 90 min at 50 °C and 64 min at 60 °C, and a better tolerance to the surfactants SDS and triton X-100. As shown in Table 1, keratinases are very diverse in properties depending on the nature of substrates and the producer organisms. They are robust enzymes as they are active over a wide range of substrates, pH, and temperature (Table 1).

## 3. Applications of keratinases

Consequently, keratinases are receiving great deal of attention because of their broad areas of applications. They have current and potential applications in waste treatment, agro-industrial, pharmaceutical and biomedical fields. Others include nanobiotechnology, leather, cosmetic and detergent industries.

### 3.1. Keratin waste management

Several microbial strains could be useful in feather waste management as they possess very remarkable feather-degrading ability (Lateef *et al*., 2010). Collagen, elastin, keratin and prion proteins generated as wastes in meat industry were efficiently degraded by the keratinolytic enzyme E77 (Zhao *et al*., 2012). Also, the decomposition of wool waste by a keratinase producing strain of *Stenotrophomonas maltophilia* has been reported (Fang *et al*., 2013). Therefore, there is potential application of keratinases as component of cocktail enzymes that can be applied in the treatment of slaughterhouse or abattoir waste stream/effluents to degrade waste components in addition to prions.

### 3.2. Animal feed production

The hydrothermal treatment reduces nutritional value, as it destroys certain essential amino acids such as methionine, lysine, histidine and tryptophan, and inability

to release some amino acids from the keratins. Thus, the use of keratinases/keratinolytic microorganisms is a good alternative. A keratinolytic strain of *B. subtilis* A1 was also reported to produce proteinous hydrolysate of high antioxidative potential from wool waste (Fakhfakh *et al*., 2013). The use of keratinase in degrading feather is more advantageous than microbial degradation, as it avoids the possibility of exposure of the users to organisms that could be pathogenic. Dietary keratinase supplementation had been reported to improve immune response, weight gain, nutrient digestibility, intestinal morphology and ecology in growing and nursery pigs (Wang *et al*., 2011a). Therefore, biodegradation of keratins serves as a veritable source of nutrient-rich feeds, nutraceuticals and feed supplements with lots of potential applications in animal husbandry.

**Table 1.** Characteristics and some applications of some keratinases from different microorganisms

| Producer organisms | Catalytic type | Optimum pH | Optimum temp. (°C) | pH stability | Temp. Stability (°C) | Mol. weight (kDa) | Substrate specificity | Inhibitors | Activators | Applications | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Bacillus halodurans* PPKS-2 | - | 11.0 | 60-70 | 7.0-13.0 | 70 | 30/66 | Feather | PMSF | - | - | Prakash *et al*. (2010b) |
| *Bacillus* sp. SH-517 | - | 7.5 | 40 | 4-9 | <50 | 51 | Casein | $Hg^{2+}$, $Ag^{2+}$, EDTA & EGTA | $K^{+}$& $Na^{+}$ | - | Jeong *et al*. (2010a) |
| *Brevibacillus* sp. AS-S10I-I | - | 12.5 | 45 | - | - | 83.2 | - | - | - | Leather & detergent industries | Rai & Mukherjee (2011) |
| *Bacillus* spp. | Serine | 10.0 | 40-50 | - | - | ~200 | Feather, gelatin | PMSF | - | Biodegradation of feather waste | Mazotto *et al*. (2011) |
| *Bacillus subtilis* NRC 3 | Metallo | 7.5/8.0 | 40/50 | 5-10 | 20-60 | - | Feather, keratin azure & azocasein | Cations | $Na^{+}$, $K^{+,}$ $Mg^{2+}$, $Ba^{2+}$& $Ca^{2+}$ | Treatment of feather waste | Tork *et al*. (2013) |
| *Paenibacillus woosongensis* TKB2 | - | 9.0 | 50 | - | - | 190.24 | Feather | - | $Mo^{+}$ | Detergent formulation | Paul *et al*. (2013b) |
| *Streptomyces albus* | Serine | 7.0 | 40 | - | - | 29-35 | Hair | PMSF, $HgCl_2$, ÞCMB, KCN, 8-Hydroxy-quinolone & cysteine | EDTA | - | Nayaka *et al*. (2013) |
| *Penicillium* spp. Morsyl | Metallo | 7.0-11.0 | 50 | 6.0-11.0 | 50-65 | 19/40 | Rice straw | PMSF, EDTA & EGTA | - | - | El-Gendy (2010) |

### 3.3. Production of nitrogen fertilizer/biofertilizer

Feather meal produced from the recycling of keratinous wastes is still applicable as a slow-release nitrogen fertilizer. Paul *et al*. (2014a) obtained feather hydrolysate through hydrolysis from *Paenibacillus woosongensis* TKB2, which induced nodule formation, and enhanced germination of seeds and growth of Bengal gram (*Cicer arietinum*). It also improved mineral elements and microbial activities in the soil, while the N, P, K and the C/N ratio was increased by 1.2-fold, thereby improving soil fertility. The incidences of free nitrogen fixers and phosphate solubilizers were increased by 2 and 5.8-fold, respectively. A keratinase-producing strain of *B. subtilis* also demonstrated plant growth-promoting and broad-spectrum antimicrobial activities, as it produced indoleacetic acid (IAA) and antifungal activities in the course of keratinase production (Jeong *et al*., 2010b).

### 3.4. Leather processing industry

Mixtures of proteases, lipases and carbohydrases have been used as biocatalysts for the dehairing process. Keratinolytic proteases without collagenolytic and elastinolytic activities are good for dehairing process (Gupta & Ramnani, 2006) as the integrity and quality

of the hides are guaranteed. Dehairing by keratinolytic proteases have proved to be the best alternative, as they selectively remove hair from the skin without damaging the skin and hair fragments. The method is easy, inexpensive and safeguards the release of toxic effluent to the environment. There are several reports of dehairing of animal skins by microbial keratinases (Rai & Mukherjee, 2011; Paul *et al*., 2013a; Chaturvedi *et al.,* 2014).

In our laboratory, the keratinase of a strain of *Bacillus safensis* LAU 13 completely dehaired goat skin within 12 h without noticeable damage to the skin, whereas incomplete dehairing with skin damage was obtained using chemical-based method (Lateef *et al.,* 2015a; Figure 1). The conflicting reports (Gupta & Ramnani, 2006; Tiwary & Gupta, 2010; Ismail *et al*., 2012; Chaturvedi *et al.,* 2014) on the involvement of collagenase and lime in the process of dehairing using keratinase may be attributable to the nature of the keratinase and the type of hide being dehaired. Nevertheless, scope exists for the application of keratinases in eco-friendly processing of leather and skin products.

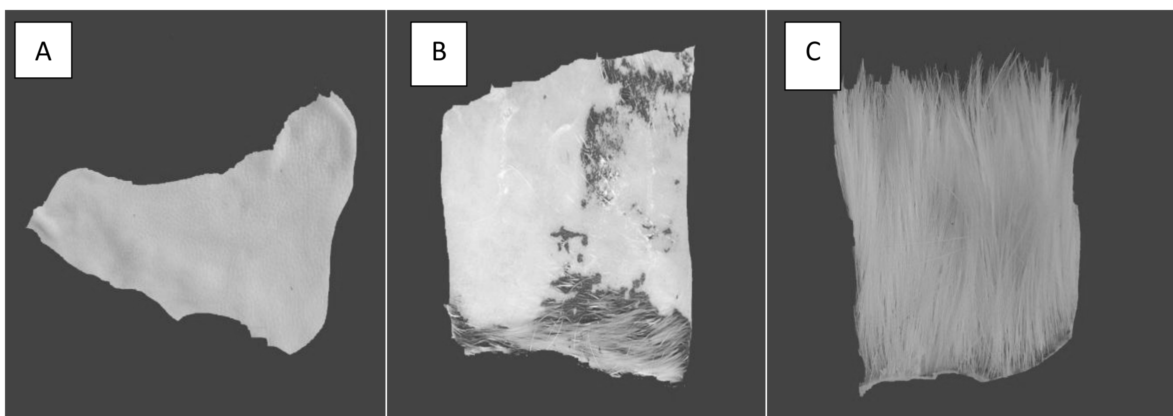


**Fig.1.** Complete dehairing of goat skin by crude keratinase from wild strain of *B. safensis* LAU 13(A), and incomplete dehairing by sodium sulfide and lime (B); Control (C).

### 3.5. Detergent industry

The keratinolytic proteases will put more to the value of proteolytic enzymes in detergent formulation due to their ability to degrade insoluble keratin and their properties like stability at high temperature and pH, activity at wide range of temperature and pH, stability in the presence of surfactants, oxidizing and bleaching agents, chelating agents and compatibility with some commercial laundry detergent (Gupta & Ramnani, 2006).

In a study, keratinase of *Paenibacillus woosongensis* TKB2 was combined with detergent and safely removed blood, fruit juice and turmeric stains from fabric (Paul *et al*., 2013b). Also, Paul *et al*. (2014b) reported the safe removal of blood, egg yolk and chocolate stains from cloths by crude keratinase. The keratinase was stable in the presence of EDTA, and the preparation of enzyme beads using 1.5 % CMC significantly improved its storage stability. In our investigation with *B. safensis* LAU 13, remarkable destaining of blood-stained cloth was achieved within 2 h of incubation (Lateef *et al*., 2015a). Thus, keratinases are important enzymes that can be used as additives in detergent formulations for efficient removal of keratinous wastes in an eco-friendly manner.

### 3.6. Textile industry

The ability of keratinases to modify silk and wool indicated their potential application in textile processing industries. Cai *et al*. (2011) treated wool and polyester-blended fabrics with crude keratinase of a strain of *Pseudomonas*, and observed that their shrink resistance and tensile strength were improved by the enzyme. The cooperative actions of cutinase, keratinase and protease have been reported to improve the wettability and anti-felting property of wool fabrics, with resultant reduction of contact angle to 66 $^{o}$, area shrinkage of 5.2 %, and acceptable strength loss of 14 % (Wang *et al*., 2011b).

### 3.7. Cosmetic and pharmaceutical applications

The non-collagenolytic keratinases are promising biocatalysts in pharmaceutical and cosmetic industries. They have been described as an ingredient in depilatory compositions for hair shaving and skin lightening agents

(Yang, 2012). Some crude keratinases have been shown to improve hair qualities such as weight, flexibility, brightness, softness and strength; thus could be applied as hair care product (Cao *et al*., 2012). Furthermore, keratinases have shown potential to degrade thickened layer of dead skin (hyperkeratosis) found in toes and fingers, thereby serving as a viable alternative to the conventional method of using salicylic acid (Gupta & Ramnani, 2006). In the same manner, keratinases are capable of skin peeling to remove acnes, which occur through the blockage of sebaceous gland by keratins (Selvam & Vishnupriya, 2012).

Prions $PrP^{Sc}$ which are infective protein molecules responsible for causing scrapie, bovine spongiform encephalopathy (BSE or "mad cow disease"), kuru, chronic wasting disease and Creutzfeldt-Jacob disease (CJD) (Caughey, 2001) have been degraded by keratinases. In addition, keratinase had been used to enhance drug delivery through topical therapy. The presence of keratinase has been reported to enhance drug penetration through the nail plate, which may be a barrier to penetration by other drugs (Mohorcic *et al*., 2007). They could also be used to disinfect medical equipment and laboratory apparatus owing to their prion protein degrading ability (Liang *et al*., 2010).

### 3.8. Other applications

Keratinous wastes can be modified by keratinases to produce biodegradable films, coatings, and glues for compostable packaging, agricultural films or edible film application (Gupta & Ramnani, 2006). Keratinases have potential for biofuel production through the anaerobic digestion of biowastes (Brandelli *et al*., 2010) and energy generation. Keratinases have been described as biocontrol molecules owing to their pesticidal and insecticidal activities (Yue *et al*., 2011).

Furthermore, keratinolytic fungi could be used to bioremediate crude oil-contaminated environments (Ulfig *et al*., 2003). Other emerging applications include the use of keratin hydrolysates to regulate haemostasis and nerve regeneration (Rouse & Van Dyke, 2010), cleaning of contact lenses (Ray, 2012), and recovery of silver from used x-ray films through the removal of the gelatin layer of used x-ray films (Seid, 2011). With the ability to hydrolyze gelatin, keratinases can also find application in food processing industries.

Recently, Revathi *et al*. (2013) discovered the potential of microbial keratinase in forming silver nanoparticles (AgNPs) through green chemistry process. We have also demonstrated the green synthesis of AgNPs using crude extracellular keratinase of *B. safensis* LAU 13 (Lateef *et al.,* 2015b, Lateef *et al.,* 2015c; Figure 2). The particles were spherical in shape with the size ranging from 5-30 nm. The FTIR spectrum of the particles showed that proteins were the capping and stabilization molecules in the synthesis of AgNPs. The particles showed effective inhibitory activity against clinical isolates of *E. coli*. Therefore, the keratinase of the strain could be used to develop an environmental friendly method for the rapid synthesis of AgNPs.

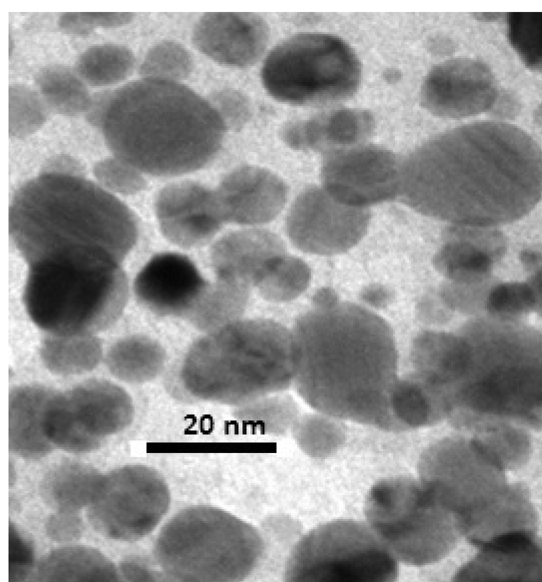


**Fig. 2.** TEM micrograph of AgNPs synthesized using crude keratinase of *B. safensis* LAU 13

## 4. Commercialization of keratinases

In spite of broad areas of applications of keratinases, very few are commercially available and typical examples being Versazyme, Valkerase, and Pure100 Keratinase (Gupta *et al.,* 2013b). It has been suggested that to enhance the commercialization of the enzymes, sector-based presentation of the applications of keratinases would be necessary for the purpose of attracting the attention of investors and entrepreneurs. The application areas are divided into three parts, viz: areas of exclusive applications, sectors where keratinases would be better alternatives to other proteases and areas that await practical documentation. World-wide production and demand for enzymes have continued to surge, with the global demand estimated to be USD 7 billion as at 2013 as per BCC Research group (Gupta *et al.,* 2013b). With the proteolytic enzymes contributing 40-60 % of industrial enzymes, keratinases with broader substrate specificity and applications would in the nearest future be dominant player in the global enzyme demand. Therefore, it is pertinent that screening for new keratinases should be application-based/driven.

## 5. Conclusion

It is evident that applications of keratinases is a growing trend that traverses industrial biotechnology, with potential applications in bioenergy, nanobiotechnology, waste recycling and management, bioremediation, leather and textile industries, food and feed technology, personal care products, medical and pharmaceutical applications, agriculture (biofertilizers, composting, plant-growth promotion, and biopesticides), biocatalysis among others. To drive these myriads of applications, search for new sources of keratinases, use of techniques to optimize enzyme yield and lowering of the production cost, and improvement of stability would be a continuous trend. The multi-functionality of keratinases will strengthen research efforts that would lead to the production and creation of novel products of biotransformation, which will continue to define bioresource utilization of keratinous wastes.

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# انزيمات الكيرتينيز: الاتجاهات الحديثة في إنتاج هذه الانزيمات وتطبيقاتها الجديدة كمواد بيولوجية حفازة متعددة الوظائف.

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## خلاصة

انزيمات الكيرتينيز Keratinases هي إنزيمات محللة للبروتين قادرة على إحداث التحلل في البروتينات الكيراتينية القاسية والغير قابلة للذوبان والموجودة في الجلد والاطراف. يتم إنتاج النزيمات من قبل الكائنات الحية الدقيقة في التراكيب الكيراتينية مثل ريشة، والشعر والصوف والأظافر، والقرن والحافر. وأغلب هذه الانزيمات محللة للبروتين الحاوي على سيرين، على الرغم من أن هناك عدد قليل جدا من التقارير حول انواع اخرى (metallokeratinases). تنشط انزيمات الكيرتينيز تحت مدى واسع من الظروف والذي يجعلها مفيدة في التدوير الحيوي للنفايات الحاوية للكيراتين لاستخدامها في الأعلاف والأسمدة كما ان لها أيضا تطبيقات محتملة في الصناعات الجلدية، ومستحضرات التجميل والمنسوجات ومواد التنظيف والتطبيقات الطبية والبيولوجية. وتمتد التطبيقات الواعدة أيضا إلى توليد الطاقة والتصنيع الصديق للبيئة للجزيئات النانوية. ونظرا لتطبيقاتها التكنولوجية الحيوية في كل مكان، استخدمت تقنيات مثل التثبيت واستراتيجيات تحقيق الامثل وهندسة البروتينات وهندسة الحمض النووي لتحسين فعاليتها وثباتها وبالتالي توسيع نطاق استخدامها التجاري. يوثق هذا الاستعراض الاتجاهات الحديثة في إنتاج هذه الانزيمات وتطبيقاتها متعددة الوظائف.