Review Article

Biotechnological Aspects and Perspective of Microbial Keratinase Production

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Keratinases are proteolytic enzymes predominantly active when keratin substrates are available that attack disulfide bridges in the keratin to convert them from complex to simplified forms. Keratinases are essential in preparation of animal nutrients, protein supplements, leather manufacture, textile processing, detergent formulation, feather meal processing for feed and fertilizer, the pharmaceutical and biomedical industries, and waste management. Accordingly, it is necessary to develop a method for continuous production of keratinase from reliable sources that can be easily managed. Microbial keratinase is less expensive than conventionally produced keratinase and can be obtained from fungi, bacteria, and actinomycetes. In this overview, the expansion of information about microbial keratinases and important considerations in keratinase production are discussed.

1. Introduction

Keratin is one of the most abundant biopolymers in the world [1]; it is a tough, fibrous, insoluble material that functions as an outer coat of human and animal organs, to prevent the loss of body fluids. Keratin is predominantly found in tissues of reptiles, birds, amphibians, and mammals. The structural component of feathers, hair, nails, horns, hooves, bones, furs, claws, hides, bird beaks, skin, wool, scales, and bristle is made up of keratin (Figure 1). α-keratins (alpha-helix) are usually found in the hair, wool, horns, nails, claws, and hooves of mammals, whereas the harder β-keratin (beta-sheets) is found in bird feathers, beaks, and claws. Keratin is also expressed in the epithelial cell types of digestive organs (liver, pancreas, intestine, and gallbladder), which include hepatocytes, hepatobiliary ductal cells, oval cells, acinar cells, enterocytes of the small intestine, colon, and goblet cells [2]. Keratin is rich in sulfur compounds with disulfide bridges, which imparts them with an insoluble nature. It also contains a variety of amino acids, predominantly cystine, lysine, proline, and serine. Keratin is hard, containing scleroprotein, while it is unreactive against most chemicals and is not digested by pepsin, trypsin, or papain [3]. Higher vertebrates, including humans, cannot digest keratinous materials. Keratin is a monomer that forms bundles of intermediate filaments that are expressed in epithelial cells that have been linked to human liver diseases. Structural details regarding keratin filaments 5 and 14 for heteromeric assembly and perinuclear organization have been reported ([4], Protein Data Bank Accession code: 3TNU; Figure 2(a)). Among different keratin filaments, K8 and K18 are important for the protection of hepatocytes [2]. The representation of K18 caspase-cleavage sites during apoptosis has been described in detail ([2], Figure 2(b)).
The major sources of keratin accumulation, which cause environmental problems, initiate from industries that use keratin as the raw material. Poultry farms are also involved in dumping of feather wastes (barbs and rachis). Indeed, 90% of feathers are keratin, and millions of kilograms of feathers are discarded to the environment annually [6]. The disposal of feathers is also accompanied by natural falling of feathers and hairs from birds during production, so it is necessary to develop methods to reduce keratin accumulation. For environmental remediation of keratin, an immediate step that has easy processing set-up with lower cost is desired. Microbial keratinase may meet these preferences, as keratinophilic fungi, bacteria, and actinomycetes naturally reside on keratin wastes. Here, we elaborated the currently available information pertaining to microbial keratinase production.

2. Keratinophilic Fungi

Keratinophilic fungi produce the proteolytic enzymes that are capable of decomposing keratinic waste materials [7]. Several keratinophilic fungi that live as parasites on keratinous materials use keratin as their carbon and nitrogen sources, multiply in an asexual manner, and produce conidia. During the process of fungal colonization, boring hyphae are produced to drill into the keratin substrate. These keratinophilic fungi include hyphomycetes and several other taxa [8]; hyphomycetes include both dermatophytic (e.g., *Microsporum* species) and nondermatophytic (e.g., *Chrysosporium* species and other genera) keratinophilic fungi [9]. The dermatophytes are mainly from the genera *Microsporum*, *Epidermophyton*, and *Trichophyton*. Keratinophilic species are
usually identified by morphological features of their macro-
and microconidia, molecular methods, and using DNA
sequence analysis [10]. Keratinophilic fungi produce sulfide
for sulphitolysis and, during this process, the disulfide bonds
of cysteine, a major amino acid in keratinous materials, are
broken down, after which the proteolytic enzymes released
by the fungi can easily cleave the keratin. During the
degradation process, the products released are cysteine, S-
sulphocysteine, cysteine acid, cysteine, and inorganic sulfate,
and the presence of these products in the culture media
indicates the occurrence of true keratinophilic fungi. Fungi
that do not show this behavior during degradation are
considered nonkeratinophilic fungi. Keratinophilic fungi are
predominantly anthropophilic (human loving) or zoophilic
(animal loving). Many keratinophilic fungi have been isolated
from soil samples due to accumulation of keratin wastes in
the soils (geophilic). Soil samples from geophilic habitats
including public beaches, agricultural areas, public parks,
gardens, and elementary schools have been found to contain
keratinophilic fungi [11–15]. Most of these studies involved an
isolation technique known as keratin-baiting, in which hair
or feathers are used for the isolation of keratinophilic fungi
[11–15]. Keratinophilic fungi isolated in countries worldwide,
including Egypt, Spain, Australia, Palestine, Kuwait, India,
Iran, and Malaysia, have been described [8].

The common isolates of keratinophilic fungi from soils include
Microsporum gypseum, M. canis, M. fulvum, M.
nanum, Trichophyton terrestris, T. ajelloi, T. mentagrophytes, T.
teridigitale, T. verrucosum, T. equinum, T. rubrum, T. teridig-
itale, T. schoenleinii, T. simii, Chrysosporium keratinophilum,
C. pannicola, C. tropicum, C. indicum, C. anum, C. lobatum,
C. evolceanui, and C. indicum. Shadzi et al. [12] have col-
clected 330 samples from thirteen elementary schools and
seven public parks and identified 214 species, among which
Chrysosporium keratinophilum was the dominant organism,
being present with a frequency of 54.2%. Anbu et al. collected
10 and 12 soil samples from poultry farms and feather
dumping locations, respectively, and recovered 34 fungal
species belonging to 19 genera. Among these, six species
are dermatophytes belonging to five genera [13]. Kachuei
et al. [15] analyzed 800 soil samples from Isfahan province
of Iran and found that 588 belong to keratinophilic fungi,
representing 73.5% of the total isolates. Furthermore, they
recovered 16 species belonging to 11 genera. Similarly, 108
soil samples from St. Kitts and 55 samples from Nevis were
shown to consist of 49 and 38 samples, respectively, positive
for keratinophilic fungi. Additionally, M. gypseum was pre-
dominantly found in 15.7 and 40% of soils of these collections
sites, respectively, followed by Chrysosporium species [14].
Molecular identification of keratinophilic fungi revealed 411
isolates from 22 genera in public park soils from Shiraz, Iran
[9]. Another study revealed that 48 soils from Jharkhand,
India, contained 10 species of keratinophilic fungi belonging
to seven genera [8]. Similarly, 500 samples collected from
zoos and parks of Ahvaz were found to contain keratinophilic
fungi [16]. In another study, 54 soil samples from different
collection sites including gardens, schools, poultry farms,
rivers, hospitals, and garbage dumping sites were found to
contain 23 species of keratinophilic fungi from 11 genera.

The abundance of samples shown to contain keratinophilic
fungi was as follows: 65% gardens, 52% schools, 43% poultry
farms, 34% garbage, 30% hospitals, and 21% rivers [7]. Based
on the above studies, it is clear that keratinophilic fungi
are ubiquitous and present in all kinds of soils and that
they are dominant in areas where humans and animals live.
In addition to the above list, keratinolytic proteins from
keratinophilic fungi were reported by Yu et al. [17], Asahi et
al. [18], and Williams et al. [19].

3. Keratin-Degrading Bacterial Isolates

Similar to the isolates of fungi, lists of bacterial strains capable
degrading keratins have been reported. Bacteria can grow
faster than fungal species and therefore have potential in
industrial applications. The advantages of fungi include easier
colonization of fungal hyphae into the harder keratin relative
to bacteria. The isolated bacterial strains known to degrade
keratin or produce the keratinase are primarily composed of
Bacillus; it includes B. subtilis and B. licheniformis [20],
although other bacteria including Gram-positive Lysobac-
ter, Nesterenkonia, Kocuria, and Microbacterium and Gram-
negative Vibrio, Xanthomonas, Stenotrophomonas, Chry-
seobacterium, Fervidobacterium, Thermoanaerobacter, and
Nesterenkonia can also degrade keratin ([21] and references
therein). Several other studies have investigated keratinase
produced by bacterial species [22–26]. Sapna and Yamini [27]
investigated the potential degradation of keratin by bacterial
strains recovered from the soil samples. Four isolates from
feather waste were recovered on milk agar plates and three
were identified as Gram-negative bacteria (Burkholderia,
Chryseobacterium, and Pseudomonas species) and one was
identified as Gram-positive strain (Microbacterium species)
[28]. Moreover, Kornilowicz-Kowalska and Bohacz [29]
reported that actinomycetes, Streptomycyes group, namely, S.
fradiae, Streptomycyes species A11, S. pactum, S. albidoflavus,
S. thermoviolaceus SD8, and S. graminofaciens, as well as
Thermoactinomycyes candidus, were capable of producing
keratinase.

4. Secretion of Microbial Keratinases

Keratinolytic enzymes are proteases known as keratinases
(EC 3.4.21/24/99.11) that can primarily be obtained from
fungi, actinomycetes, and bacteria [29]. Fungal keratinases
can be easily obtained by secretion, and their low cost makes
them preferable over bacterial keratinases in some cases,
even though the fungi grow slower and the recovery of
keratinase from fungi has been reported for several decades.
The availability of several strains that are capable of producing
keratinase makes the situation to select efficient keratinase
producers an important step. Screening microbial enzymes
is essential in the selection process, and the chosen enzymes
should be less expensive, eco-friendly, and efficient. Both
keratinophilic fungi and nonkeratinophilic fungi can produce
keratinases, but the difference is the rate of production,
which is higher in the former case. Several methods have
been proposed to screen proteolytic (including keratinolytic)
enzymes, including keratin-baiting, plate screening, spectrophotometric methods, and sequence-based amplification. Jeevana Lakshmi et al. [30] identified feather-degrading bacteria using the 16S rDNA sequence. Among the aforementioned methods, the plate-clearing assay is one of the popular methods due to displaying visual results, as well as being less expensive and easier than other methods (Figure 3). The keratin-baiting method is used for the initial screening and isolation of keratinolytic species. In this method, any keratin source can be the bait; hair and feathers are routinely in use [11, 13]. Even though the pour plate method can be used to isolate the keratinophilic microbes as an alternate, keratin-baiting is also commonly applied because it enables the direct selection of keratinophilic species on the substrate.

5. Optimized Conditions for Microbial Keratinases

Once microbes are isolated, they can be further cultivated on suitable artificial growth media under optimal conditions to obtain excess production of keratinase. Sabouraud’s dextrose is commonly used to grow keratinophilic fungi due to its suitability [11, 13, 16]. Usually keratinophilic fungi will take a longer time to degrade the keratin (in weeks). Using the hair-baiting technique, Gugnani et al. [14] found that 4 to 8 weeks were required to observe keratinophilic fungal growth.

Kumar et al. [8] isolated keratinophilic fungi after 2 to 4 weeks of incubation, while Mahmoudabadi and Zarrin [16] found that 4 to 5 weeks are necessary to grow. In such cases, optimal growth was found to occur at room temperature. It has also been reported that keratinophilic fungi are able to degrade 40% of keratin after 8 weeks, while less than half (<20%) of that amount can be degraded in the case of nonkeratinophilic fungi [31].

It has been reported that most keratinophilic microbes thrive well under neutral and alkaline pH, the range being 6.0 to 9.0 [32]. Most keratinophilic fungi are mesophiles, although *M. gypseum* and some species of *Chrysosporium* are thermotolerant ([129] and references therein). It has been reported that temperatures of 28°C to 50°C favor keratinase production by most bacteria, actinomycetes, and fungi, while 70°C favors its production by *Thermoanaerobacter* and *Fervidobacterium* species [33–35]. Optimal keratinase production by *Chrysosporium keratinophilum* occurs at 90°C and its half-life is 30 min [36], whereas the thermophile *Fervidobacterium islandicum* AW-1 has an optimum of 100°C and a half-life of 90 min [35].

The complete optimized conditions for microbial keratinases production are described in detail elsewhere [37]. Under optimal condition, keratinophilic fungi, *Scopulariopsis brevicaulis* and *Trichophyton mentagrophytes*, result in keratinase activity to the levels 3.2 and 2.7 Keratinase
Unit (KU)/mL with the ability to degrade 79 and 72.2% of chicken feathers, respectively [38]. Matikeviciene et al. [20] have shown keratinase activity of 152 KU/mL after 24 h of incubation using Bacillus species with optimal media. Higher amounts of keratinase were reported by Kanchana [3] at 37°C for 72 h in medium containing feather meal and 0.025% yeast extract at a pH 7.0 under submerged culture. Laba and Rodziewicz [39] optimized the conditions for keratinolytic feather-degrading ability of Bacillus polymyxa and B. cereus. Additionally, Sivakumar et al. [40] recently optimized the culture conditions for the production of keratinase from Bacillus cereus TSI. Using dimeric keratinase obtained from Bacillus licheniformis ER-15 complete degradation was achieved within 8 h at pH 8 and 50°C. In this case, 25 g of chicken feathers was degraded with 1200 KU [41].

6. Purification of Keratinases

In addition to the higher keratinase production under optimal conditions, purification of keratinase is necessary for further industrial applications to hasten the efficiency of keratinase action. Molyneux [42] attempted to isolate keratinase from a bacterial source. In other cases, with the purified keratinases, several sizes were reported in the apparent molecular weight range of 27 to 200 kDa from different strains of bacteria and fungi ([29] and references therein). However, Kim et al. [43] reported recovery of keratinase with a molecular weight of 440 kDa. Purified enzymes including keratinases can be obtained using different methodologies. The most common strategy is to purify the enzymes by precipitation followed by column chromatography. Keratinase with a molecular mass of 35 kDa was purified from feather-degrading bacterium using ammonium sulphate precipitation followed by ion-exchange (DEAE-Sepharose) and gel-filtration (Sephadex G-75). The purified keratinase was found to have thermostolerant and showed high specific activity [44]. Using a similar strategy, Zhang et al. [45] purified the alkaline keratinase from Bacillus species and identified keratinase of 27 kDa using MALDI-TOF-MS. Anbu et al. [5] isolated keratinase with a molecular weight of 39 kDa from the poultry farm isolate, Scopulariopsis brevicaulis, and found that this keratinase had a serine residue near the active site. Keratinase with a size of 41 ± 1 kDa and activity under the optimal conditions at pH 9.0 and 50°C was isolated from Bacillus megaterium. This enzyme was also found to have a serine active site and to be inhibited by PMSF [46]. Based on the pH adaptation nature of the keratinase, the column matrix and method of purification can be desired while varying the elution profile (Figure 4). In addition, keratinase purification can also be accomplished with greater efficiency by immunoprecipitation when the appropriate anti-keratinase antibody is available. Similarly, immunochromatography technique can be implemented using anti-keratinase antibody for the efficient purification of keratinase. Purified keratinases from diverse species have displayed higher stability under varied condition (Table 1).

7. Acceleration of Microbial Keratinase Production

Following the optimization of the basic conditions for keratinase production and purification, it is necessary to accelerate overproduction of keratinase. This can be accomplished by recombinant DNA technology and statistical optimization. Sequences for both the substrate-keratin and the enzyme-keratinase have been proposed. The primary sequences of the keratin involved in its recombinant production were found by
### Table 1: Keratinases from different species for various applications.

| Species                              | Optimal condition (pH) | Aim(s) of study                     | Reference |
|--------------------------------------|------------------------|-------------------------------------|-----------|
| **Fungi**                            |                        |                                     |           |
| Aspergillus oryza                    | 8.0                    | Purification and characterization    | [47]      |
| Doratomyces microsporum              | 8.0–9.0                | Comparative analysis                | [48]      |
| Paecilomyces marquandii              | 8.0                    | Comparative analysis                | [48]      |
| Trichophyton rubrum                  | 8.0                    | Purification and characterization    | [18]      |
| Microsporum gyipseum                 | 8.0                    | Secretion of keratinase             | [49]      |
| Scopulariopsis brevicaulis           | 8.0                    | Dehairing process                   | [5]       |
| Myrothecium verrucaria               | 8.3                    | Feather degradation                 | [50]      |
| Chrysosporium keratinophilum         | 9.0                    | Stable keratinase                   | [36]      |
| Trichoderma atroviride               | 8.0–9.0                | Feather degradation                 | [51]      |
| **Bacteria**                         |                        |                                     |           |
| Clostridium sporogenes               | 8.0                    | Novel keratinolytic activity        | [52]      |
| Microbacterium arborescens           | 7.0                    | Feather degradation                 | [53]      |
| Fervidobacterium islandicum          | 9.0                    | Feather degradation                 | [35]      |
| Kytococcus sedentarius               | 7.0–7.5                | Feather degradation                 | [54]      |
| Stenotrophomonas maltophilia         | 7.8                    | Purification and characterization    | [55]      |
| Kocuria rosea                        | 7.5                    | Feather degradation                 | [56]      |
| Xanthomonas maltophilia              | 8.0                    | Purification and characterization    | [57]      |
| Streptomyces thermoviolaceus         | 8.0                    | Feather degradation                 | [58]      |
| Bacillus pumilus                     | 10.0                   | Purification and characterization    | [59]      |
| Thermoanaerobacter keratinophilum    | 8.0                    | Isolation of keratinophilic species | [34]      |

Hanukoglu and Fuchs [60, 61] and denoted by type I and type II. Later, several amino acids sequences for keratinase were revealed. The amino acid sequence of keratinase from *Bacillus licheniformis* and other species is available in data bank ([62], e.g., accession code AAB34259). Similarly, the full length of keratin sequences from *Homo sapiens* has been reported ([63], accession code P04264). For the large-scale preparation of keratinase, recombinant DNA technology would yield a large amount of overexpressed enzyme. Recombinant or other keratinases purified using conventional methods have great potential for applications in industrial processes such as dehairing. For example, Anbu et al. [5] have accomplished dehairing using purified keratinase from the keratinophilic fungi, *Scopulariopsis brevicaulis* (Figure 5).

The production levels of any given enzyme can also be improved severalfold using statistical modeling studies. There are different formulations of statistical calculations with basic formulae that have been described. Some basic models for optimization are given in Figure 6, which shows a response surface methodology perturbation plot and mixture trace plot. One of the basic models, the Box-Behnken design, is related to experimental variables by the response equation:

\[
Y = R_0 + \sum_{i=1}^{k} R_i X_i + \sum_{i=1}^{k} R_{ij} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} R_{ij} X_i X_j + \varepsilon.\tag{2}
\]

The variables and other parameters have been described previously in detail [64]. Using a statistical optimization model, Harde et al. [65] optimized the keratinase production of *Bacillus subtilis* NCIM 2724. These authors used one-factor-at-a-time optimization and an orthogonal array design. Recently, Shankar et al. [66] used response surface methodology, for the optimization of keratinase production by *Bacillus thuringiensis*. Using this design experiment, they compared the actual experimental and predicted calculated values and found that pH 10 and 50°C with 1% mannitol were ideal for keratinase production from *B. thuringiensis*. Similarly, Ramnani and Gupta [67] optimized the medium composition for the production of keratinase from *B. licheniformis* RG1 using response surface methodology. In another study, *B. cereus* was used for the study to optimize keratinase production [68]. Using the Box-Behnken design experiments, Anbu et al. [5] optimized the activity of purified keratinase from *Scopulariopsis brevicaulis* and achieved 100% activity with 5 mM CaCl₂ at pH 8.0 and 40°C. Similarly, production of keratinase by *Scopulariopsis brevicaulis* and *Trichophyton mentagrophytes* has also been optimized using Box-Behnken design experiments by Anbu et al. [38, 69].
8. Sensing Keratinases

In the above sections, various aspects regarding the conditions necessary for keratinase to degrade keratin are provided. However, detection strategies are also important for future applications of keratinase. Detection of keratinase or other biomolecules and their interactive analyses with binding partners can be accomplished using biosensors. Biosensors consist of a physicochemical detector and a biological component, enabling binding events to be transduced, thereby allowing detection of very small amounts of target biomolecules (keratinase). Sensors are broadly

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**Figure 5:** Dehairing using microbial keratinase (2 KU/mL) produced by *Scopulariopsis brevicaulis* (source from [5]). The purified keratinase was sprayed on the flesh side of the skin and then folded and incubated for 30 days. Every 3 days of interval, the dehairing ability was examined.

**Figure 6:** Basic strategy for statistical optimization of keratinase. (a) Response surface methodology perturbation plot; (b) mixture trace plot. Response surface methodology is a collection of statistical techniques for designing experiments, building models, and evaluating the effective factors. It is an efficient statistical technique for optimization of multiple variables to predict best performance conditions with minimum number of experiments.
classified as electrochemical, electrical, optical and mass-sensitive, chemiluminescence, fluorescence, quantum dot-based, colorimetric, and mass spectroscopic detections. Different sensing surfaces can be adopted for the detection of keratinases. Developing sensing strategies for the detection of keratinase favors the analysis of keratinase from mixtures of a given sample.

Generally, gold- or silica-based sensing surfaces have been used to analyse the biomolecules [70–77]. To capture keratinase on these surfaces, appropriate tags can chemically modify keratinase. Thiol-modification of keratinase can enable its attachment onto the surface of gold or modification of the sensing surface with the COOH-terminal for ultimate attachment to amines on keratinase. Similarly, in the case of silica, surfaces must be chemically modified using amino-coupling agent followed by suitable tags, which can couple an amino group on the keratinase. In short, both gold and coupling agent followed by suitable tags, which can couple attachment to amines on keratinase. Similarly, in the case of enable its attachment onto the surface of gold or modification cally modify keratinase. Thiol-modification of keratinase can been used to analyse the biomolecules [70–77]. To capture keratinase on these surfaces, appropriate tags can chemi- be functionalized.

9. Perspectives

Keratin, which is one of the most abundant hard materials in soil, is difficult to degrade under natural conditions. However, microbial degradation is an easier and less expensive method for conversion of these products to useful end products. Several methods to improve keratinase production have been suggested, and keratinase has been overexpressed, successfully purified, and applied to several industrial applications. In addition, additional developments have been implemented in keratinase research recently [78, 79]. However, there is currently no highly sensitive system available for the detection of keratinases. In addition, use of recombinant keratinase chimeras has the potential to generate efficient keratinase and needs to be improved. Development of more efficient methods for the production and detection of keratinase will hasten its application to industries and environmental waste management.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] A. L. Martínez-Hernández and C. Velasco-Santos, “Keratin fibers from chicken feathers: structure and advances in polymer composites,” in Keratin: Structure, Properties and Applications, R. Dullaart and J. Mousqués, Eds., pp. 149–211, Nova Science Publishers, 2012.
[2] A. M. Fortier and M. Cadrin, “Simple epithelial keratins k8 and k18: from structural to regulatory protein,” in Keratin: Structure, Properties and Applications, R. Dullaart and J. Mousqués, Eds., pp. 1–35, Nova Science Publishers, 2012.
[3] R. Kanchana, “Farm waste recycling through microbial keratinases,” Journal of Applied Sciences in Environmental Sanitation, vol. 7, no. 2, pp. 103–108, 2012.
[4] C.-H. Lee, M.-S. Kim, B. M. Chung, D. J. Leahy, and P. A. Coulombe, “Structural basis for heteromeric assembly and perinuclear organization of keratin filaments,” Nature Structural and Molecular Biology, vol. 19, no. 7, pp. 707–715, 2012.
[5] P. Anbu, S. C. B. Gopinath, A. Hilda, T. Lakshmi Priya, and G. Annadurai, “Purification of keratinase from poultry farm isolate-Scopulariopsis brevicaulis and statistical optimization of enzyme activity,” Enzyme and Microbial Technology, vol. 36, no. 5–6, pp. 639–647, 2005.
[6] M. N. Acda, “Waste chicken feather as reinforcement in cement-bonded composites,” Philippine Journal of Science, vol. 139, no. 2, pp. 161–166, 2010.
[7] M. A. Ganaie, S. Sood, G. Rizvi, and T. A. Khan, “Isolation and identification of keratinophilic fungi from different soil samples in Jhansi City (India),” Plant Pathology Journal, vol. 9, no. 4, pp. 194–197, 2010.
[8] R. Kumar, R. Mishra, S. Maurya, and H. B. Sahu, “Isolation and identification of keratinophilic fungi from garbage waste soils of Jharkhand region of India,” European Journal of Experimental Biology, vol. 3, no. 3, pp. 600–604, 2013.
[9] H. C. Gugnani, “Non-dermatophytic filamentous keratinophilic fungi and their role in human infections,” in Biology of Dermato-phyes and Other Keratinophilic Fungi, R. K. S. Kushwaha and J. Guarro, Eds., pp. 109–114, Revista Iberoamericana de Micologia Apartado, Bilbao, Spain, 2000.
[10] P. K. Ramesh and A. Hilda, “Incidence of keratinophilic fungi in the soil of primary schools and public parks of Madras city, India,” Mycopathologia, vol. 143, no. 3, pp. 139–145, 1998.
[11] S. Shadzi, M. Chadeganipour, and M. Alimoradi, “Isolation of keratinophilic fungi from elementary schools and public parks in Isfahan, Iran,” Mycoses, vol. 45, no. 11-12, pp. 496–499, 2002.
[12] P. Anbu, A. Hilda, and S. C. B. Gopinath, “Keratinophilic fungi of poultry farm and feather dumping soil in Tamil Nadu, India,” Mycopathologia, vol. 158, no. 3, pp. 303–309, 2004.
[13] C. C. Gugnani, S. Sharma, B. Gupta, and S. Gaddam, “Prevalence of keratinophilic fungi in soils of St. Kitts and Nevis,” Journal of Infection in Developing Countries, vol. 6, no. 4, pp. 347–351, 2012.
[14] R. Kanchana, “Farm waste recycling through microbial keratinases,” Journal of Applied Sciences in Environmental Sanitation, vol. 7, no. 2, pp. 103–108, 2012.
[15] A. Z. Mahmoudabadi and M. Zarrin, “Isolation of dermatophytes and related keratinophilic fungi from the two public
parks in Ahvaz," *Jundishapur Journal of Microbiology*, vol. 1, no. 1, pp. 20–23, 2008.

[17] R. J. Yu, S. R. Harmon, and F. Blank, "Isolation and purification of an extracellular keratinase of *Trichophyton mentagrophytes*," *Journal of Bacteriology*, vol. 96, no. 4, pp. 1435–1436, 1968.

[18] M. Asahi, R. Lindquist, K. Fukuyama, G. Apodaca, W. L. Epstein, and J. H. McKerrow, "Purification and characterization of major extracellular proteinases from *Trichophyton rubrum*," *Biochemical Journal*, vol. 232, no. 1, pp. 139–144, 1985.

[19] C. M. Williams, C. S. Richter, J. M. MacKenzie Jr., and J. C. H. Shih, "Isolation, identification and characterization of a feather-degrading bacterium," *Applied and Environmental Microbiology*, vol. 56, no. 6, pp. 1509–1515, 1990.

[20] V. Matikevičienė, D. Masiliuniene, and S. Grigiskis, "Degradation of keratin containing wastes by bacteria with keratinolytic activity," in *Proceedings of the 7th International Scientific and Practical Conference*, vol. 1, pp. 284–289, 2009.

[21] R. Gupta and P. Ramnani, "Microbial keratinases and their prospective applications: an overview," *Applied Microbiology and Biotechnology*, vol. 70, no. 1, pp. 21–33, 2006.

[22] H. Korkmaz, H. Hürr, and S. Dincer, "Dincer, Characterization of alkaline keratinase of *Bacillus licheniformis* strain HK-1 from poultry waste," *Annals of Microbiology*, vol. 54, no. 2, pp. 201–211, 2004.

[23] M. M. Hoq, K. A. Z. Siddiquee, H. Kawasaki, and T. Seki, "Keratinolytic activity of some newly isolated *Bacillus* species," *Journal of Biological Sciences*, vol. 5, no. 2, pp. 193–200, 2005.

[24] G.-W. Nam, D.-W. Lee, H.-S. Lee et al., "Native-feather degradation by a mutant strain of *Bacillus polymyxa* and *Bacillus cereus*," *Journal of Biophysics*, vol. 5, no. 1, pp. 60–67, 2005.

[25] M. Cortez, J. Contiero, C. J. B. de Lima, R. B. Lovaglio, and R. Monti, "Characterization of a feather degrading by *Bacillus amyloliquefaciens* protease: a new strain," *World Journal of Agricultural Sciences*, vol. 4, no. 5, pp. 648–656, 2008.

[26] R. Sapna and V. Yamin, "Study of keratin degradation by some potential bacterial isolates from soil," *Journal of Soil Science*, vol. 1, no. 1, pp. 1–3, 2011.

[27] A. Riffel and A. Brandelli, "Keratinolytic bacteria isolated from feather waste," *Brazilian Journal of Microbiology*, vol. 37, no. 3, pp. 395–399, 2006.

[28] T. Kornilowicz-Kowalska and J. Bohacz, "Biodegradation of keratin waste: theory and practical aspects," *Waste Management*, vol. 31, no. 8, pp. 1689–1701, 2011.

[29] P. Jeevana Lakshmi, C. M. Kumari Chitturi, and V. V. Lakshmi, "Efficient degradation of feather by keratinase producing *Bacillus sp.*," *International Journal of Microbiology*, vol. 2013, Article ID 608321, 7 pages, 2013.

[30] J. Kunert, "Physiology of keratinophilic fungi," in *Revista Iberoamericana de Micología*, R. K. S. Kushawaha and J. Guvar, Eds., pp. 77–85, Búlba, Spain, 2000.

[31] N. Jain and M. Sharma, "Biodiversity of keratinophilic fungal flora in university campus, Jaipur, India," *Islamic Journal of Public Health*, vol. 41, no. 11, pp. 27–33, 2012.

[32] A. B. Friedricht and G. Antranikian, "Keratin degradation by *Fervidobacterium pennavorans*, a novel thermophilic anaerobic species of the order thermostogales," *Applied and Environmental Microbiology*, vol. 62, no. 8, pp. 2875–2882, 1996.

[33] S. Riessen and G. Antranikian, "Isolation of *Thermoanaerobacter keratinophilus* sp. nov., a novel thermophilic, anaerobic bacterium with keratinolytic activity," *Extremophiles*, vol. 5, no. 6, pp. 399–408, 2001.

[34] G.-W. Nam, D.-W. Lee, H.-S. Lee et al., "Native-feather degradation by *Fervidobacterium islandicum* AW-1, a newly isolated keratinase-producing thermophilic anaerobe," *Archives of Microbiology*, vol. 178, no. 6, pp. 538–547, 2002.

[35] I. N. S. Dozie, C. N. Okeke, and N. C. Unaeze, "A thermostable, alkaline-active, keratinolytic proteinase from *Chryosporium keratinophilum*," *World Journal of Microbiology and Biotechnology*, vol. 10, no. 5, pp. 563–567, 1994.

[36] A. Brandelli, D. J. Daroti, and A. Riffel, "Biochemical features of microbial keratinases and their production and applications," *Applied Microbiology and Biotechnology*, vol. 85, no. 6, pp. 1735–1750, 2010.

[37] P. Anbu, S. C. B. Gopinath, A. Hilda, N. Mathivanan, and G. Kannadurai, "Secretion of keratinolytic enzymes and keratinolysis by *Scopulariopsis brevicaulis* and *Trichophyton mentagrophytes*: regression analysis," *Canadian Journal of Microbiology*, vol. 52, no. 11, pp. 1060–1069, 2006.

[38] W. Laba and A. Rodziewicz, "Keratinolytic potential of feather-degrading *Bacillus polymyxa* and *Bacillus cereus*," *Polish Journal of Environmental Studies*, vol. 19, no. 2, pp. 371–378, 2010.

[39] T. Sivakumar, T. Shankar, V. Thangapand, and V. Ramasubram, "Optimization of cultural condition for keratinase production using *Bacillus cereus* TSI," *Insight Microbiology*, vol. 3, no. 1, pp. 1–8, 2013.

[40] E. Tiwary and R. Gupta, "Rapid conversion of chicken feather to feather meal using dimeric keratinase from *Bacillus licheniformis* ER-15," *Journal of Bioprocessing & Biotechniques*, vol. 2, no. 4, Article ID 1000123, 2012.

[41] G. S. Molyneux, "The digestion of wool by a keratinolytic *Bacillus*," *Australian Journal of Biological Sciences*, vol. 12, no. 3, pp. 274–281, 1959.

[42] J.-S. Kim, L. D. Klusken, W. M. de Vos, R. Huber, and J. Van Der Oost, "Crystal structure of fivoldysis from *Fervidobacterium pennivorans*, a keratinolytic enzyme related to subtilisin," *Journal of Molecular Biology*, vol. 335, no. 3, pp. 787–797, 2004.

[43] W. Sunternsriuk, J. Tongjun, P. Onnim et al. "Purification and characterisation of keratinase from a thermotolerant feather-degrading bacterium," *World Journal of Microbiology and Biotechnology*, vol. 21, no. 6–7, pp. 1111–1117, 2005.

[44] B. Zhang, Z. W. Sun, D. D. Jiang, and T. G. Niu, "Isolation and purification of alkaline keratinase from *Bacillus* sp. 50-3," *African Journal of Biotechnology*, vol. 8, no. 11, pp. 2598–2603, 2009.

[45] V. Salabab, F. N. Niyonzima, and S. S. More, "Isolation, partial purification and characterization of keratinase from *Bacillus magaterium*," *International Research Journal of Biological Sciences*, vol. 22, no. 2, pp. 13–20, 2013.

[46] A. M. Farag and M. A. Hassan, "Purification, characterization and immobilization of a keratinase from *Aspergillus oryzae*," *Enzyme and Microbial Technology*, vol. 34, no. 2, pp. 85–93, 2004.

[47] H. Gradišar, J. Friedrich, I. Krizaj, and R. Jerala, "Similarities and specificities of fungal keratinolytic proteases: comparison of keratinases of *Paecilomyces marquandii* and *Doratomyces microsporus* to some known proteases," *Applied and Environmental Microbiology*, vol. 71, no. 7, pp. 3420–3426, 2005.
[49] K. C. Raju, U. Neogi, R. Saumya, and N. R. Goud, “Studies on extra cellular enzyme keratinase from dermatophyte Microsporum gypseum,” International Journal of Biological Chemistry, vol. 1, no. 3, pp. 174–178, 2007.

[50] F. G. Moreira-Gasparin, C. G. M. de Souza, A. M. Costa et al., “Purification and characterization of an efficient poultry feather degrading-protease from Myrothecium verrucaria,” Biodegradation, vol. 20, no. 5, pp. 727–736, 2009.

[51] L. Cao, H. Tan, Y. Liu, X. Xue, and S. Zhou, “Characterization of a novel keratinolytic Trichoderma atroviride strain F6 that completely degrades native chicken feather,” Letters in Applied Microbiology, vol. 46, no. 3, pp. 389–394, 2008.

[52] E. Ionata, F. Canganella, G. Bianconi et al., “A novel keratinolytic keratinase from Clostridium sporogenes bv. pennavorans bv. nov., a thermotolerant organism isolated from solfataric muds,” Microbiological Research, vol. 163, no. 1, pp. 105–112, 2008.

[53] R. C. S. Thys, F. S. Lucas, A. Riffel, P. Hein, and A. Brandelli, “Characterization of a protease of a feather-degrading Microbacterium species,” Letters in Applied Microbiology, vol. 39, no. 2, pp. 181–186, 2004.

[54] C. M. Longshaw, J. D. Wright, A. M. Farrell, and K. T. Holland, “Kytococcus sedentarius, the organism associated with keratinolysis, produces two keratin-degrading enzymes,” Journal of Applied Microbiology, vol. 93, no. 5, pp. 810–816, 2002.

[55] Z.-J. Cao, Q. Zhang, D.-K. Wei et al., “Characterization of a novel Stenotrophomonas isolate with high keratinase activity and purification of the enzyme,” Journal of Industrial Microbiology and Biotechnology, vol. 36, no. 2, pp. 181–188, 2009.

[56] L. Vidal, P. Christen, and M. N. Coello, “Feather degradation of a thermotolerant organism isolated from solfataric muds,” United States of America, vol. 51, no. 3, pp. 174–178, 2007.

[57] L. D. Johnson, W. W. Idler, X.-M. Zhou, D. R. Roop, and P. M. Steinert, “Structure of a gene for the human epidermal 67-kDa keratin,” Proceedings of the National Academy of Sciences of the United States of America, vol. 82, no. 7, pp. 1896–1900, 1985.

[58] S. C. B. Gopinath, P. Anbu, T. Lakshmi, and A. Hilda, “Strategies to characterize fungal lipases for applications in medicine and dairy industry,” BioMed Research International, vol. 2013, Article ID 154549, 10 pages, 2013.

[59] S. M. Harde, I. B. Bajaj, and R. S. Singhal, “Optimization of fermentative production of keratinase from Bacillus subtilis NCIM 2724,” Agriculture, Food and Analytical Microbiology, vol. 1, no. 1, pp. 54–65, 2011.

[60] T. Shankar, P. Thangamathi, S. Sathiya, and T. Sivakumar, “Statistical optimization of keratinase production by Bacillus thuringiensis,” Journal of Global Biosciences, vol. 3, no. 2, pp. 477–483, 2014.

[61] P. Rammani and R. Gupta, “Optimization of medium composition for keratinase production on feather by Bacillus licheniformis RGI using statistical methods involving response surface methodology,” Biotechnology and Applied Biochemistry, vol. 40, no. 2, pp. 191–196, 2004.

[62] S. T. Shankar, T. Shankar, P. Vijayabaskar, and V. Rameshmanian, “Statistical optimization of keratinase production by Bacillus cereus,” Global Journal of Biotechnology and Biochemistry, vol. 6, no. 4, pp. 197–202, 2011.

[63] P. Anbu, S. C. B. Gopinath, A. Hilda, T. Lakshmi, and G. Annadurai, “Optimization of extracellular keratinase production by poultry farm isolate Scopulariopsis brevicaulis,” Bioresource Technology, vol. 98, no. 6, pp. 1298–1303, 2007.

[64] S. C. B. Gopinath, K. Awazu, J. Tominaga, and P. K. R. Kumar, “Monitoring biomolecular interactions on a digital versatile disk: a BioDVD platform technology,” ACS Nano, vol. 2, no. 9, pp. 1885–1895, 2008.

[65] S. C. B. Gopinath, R. Kumaresan, K. Awazu et al., “Evaluation of nucleic acid duplex formation on gold over layers in biosensor fabricated using Czochralski-grown single-crystal silicon substrate,” Analytical and Bioanalytical Chemistry, vol. 398, no. 2, pp. 751–758, 2010.

[66] S. C. B. Gopinath, K. Awazu, and M. Fujimaki, “Waveguide-mode sensors as aptasensors,” Sensors, vol. 12, no. 2, pp. 2136–2151, 2012.

[67] S. C. B. Gopinath, K. Awazu, M. Fujimaki, K. Shimizu, and T. Shima, “Observations of immuno-gold conjugates on influenza viruses using waveguide-mode sensors,” PLoS ONE, vol. 8, no. 7, Article ID e69121, 2013.

[68] T. S. Dhahi, U. Hashim, N. M. Ahmed, and H. Nazma, “Fabrication and characterization of gold nano-gaps for ssDNA immobilization and hybridization detection,” Journal of New Materials for Electrochemical Systems, vol. 14, no. 3, pp. 191–196, 2011.

[69] M. E. Ali, U. Hashim, S. Mustafa et al., “Nanoparticle sensor for label free detection of swine DNA in mixed biological samples,” Nanotechnology, vol. 22, no. 19, Article ID 195503, 2011.

[70] M. E. Ali, U. Hashim, S. Mustafa, Y. B. Che Man, and K. N. Islam, “Gold nanoparticle sensor for the visual detection of pork adulteration in meatball formulation,” Journal of Nanomaterials, vol. 2012, Article ID 103607, 7 pages, 2012.

[71] T. Lakshmi, M. Fujimaki, S. C. B. Gopinath, K. Awazu, Y. Horiguchi, and Y. Nagasaki, “A high-performance waveguide-mode biosensor for detection of factor IX using PEG-based blocking agents to suppress non-specific binding and improve sensitivity,” Analyst, vol. 138, no. 10, pp. 2863–2870, 2013.

[72] D. J. Daroit and A. Brandelli, “A current assessment on the production of bacterial keratinases,” Critical Reviews in Biotechnology, vol. 34, no. 4, pp. 372–384, 2014.

[73] S. Gupta and R. Singh, “Hydrolyzing proficiency of keratinases in feather degradation,” Indian Journal of Microbiology, vol. 54, no. 4, pp. 466–470, 2014.