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An Overall Review On Microbial Degradation Of Keratin Containing Waste Products

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Abstract
Keratin is a structural fibrous protein found in feathers, hair, wool, horns, hooves, and similar materials. It emphasizes the environmental challenges associated with keratin waste, as it is often discarded through dumping, burial, or incineration, leading to public health concerns and contributing to greenhouse gas emissions. Keratin's molecular structure makes it exceptionally durable and resistant to degradation. The protein consists of densely packed polypeptide chains, enriched with cysteine residues that form strong intermolecular bonds, giving keratin its toughness and tensile strength. As a result, keratin waste is considered recalcitrant, meaning it resists breakdown through natural processes. The review highlights that physical and chemical methods of keratin degradation have limitations, such as being inefficient, expensive, or environmentally harmful. Instead, the focus shifts to biological methods for processing keratin waste. Soil is identified as the natural habitat where diverse microbial populations (mainly bacteria and fungi) thrive, many of which possess the ability to degrade keratin. These microorganisms produce specialized enzymes, called keratinases, which break down the tough keratin structure. The review further explores the potential and applications of keratinase enzymes in various industries, including waste management, textile, and leather industries. It discusses the benefits of using biological methods, as they are more environmentally friendly and sustainable compared to traditional chemical approaches. Additionally, the review touches on the growing interest in the recombinant production of keratinase. Genetic engineering techniques are being used to enhance the production, bioavailability, and efficiency of keratinase enzymes. The recombinant approach could significantly improve the scalability of keratin waste degradation, making it more feasible for industrial applications. In conclusion, the review underlines the importance of exploring biological methods, especially microbial degradation, as a promising alternative for keratin waste management, with a special focus on the role of keratinases and their recombinant production.
Keywords: *Keratin degradation; Keratinase; Microbial biotechnology; Keratinous waste; Bioremediation.*

INTRODUCTION

Keratin is a fibrous structural protein that is found only in cells of chordates like vertebrates, amphioxus, and urochordates. These include mammals, fishes, reptiles, birds, and amphibians. Apart from chitin, keratin is the only other tough protein that protects the epithelial cells and gives strength to some organs (Anne Marie Helmenstine. 2018). Industries like poultry farms, slaughterhouses, leather, wool, and hair salons are the prime sources of generating keratin-containing biomass (Figure 1), although, poultry industries are vital sources for producing tons of keratin waste (Kreplak et al., 2001). This waste generated is the natural source of keratin used for various commercial applications such as fertilizer, dusters, bedding materials, animal feed, and cosmetic industries. (Papadopoulos et al., 1986; Swati and Arun. 2016). The key producers of keratin waste include the United States of America, China, India, and Brazil. Around 8.5 billion tons of poultry feathers are generated worldwide annually from the poultry processing plant, of which India's contribution alone is 350 million tons.

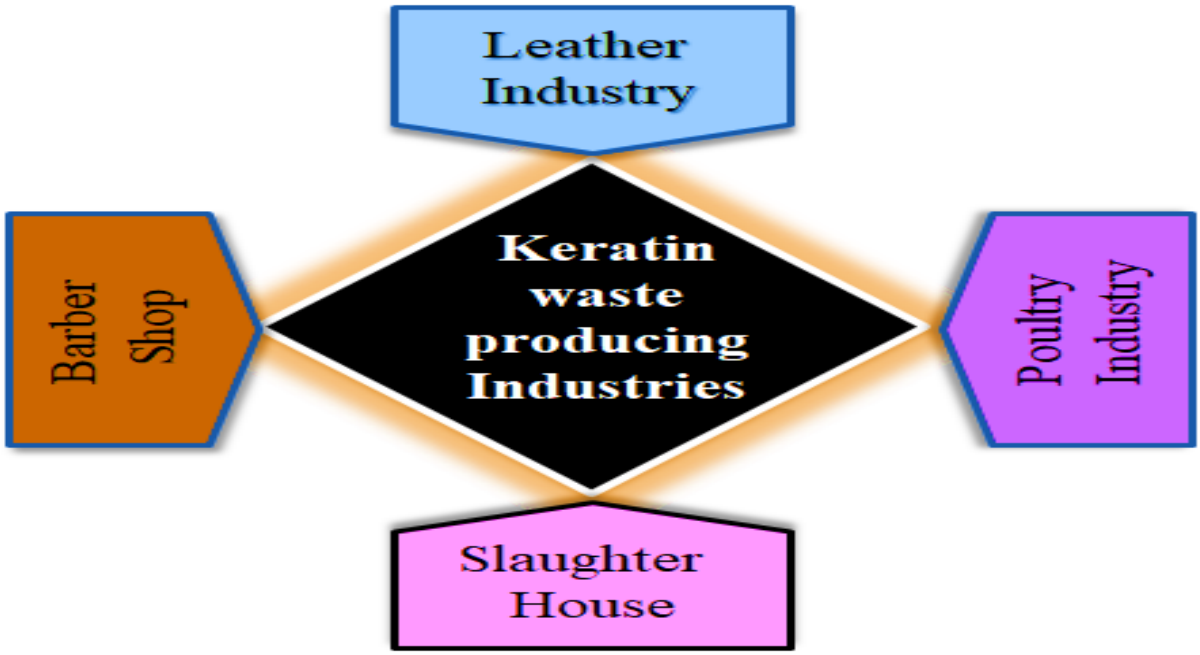

Figure 1. Key source of keratin waste from various industries.

Keratin wastes from various industries are considered as environmental pollutants. These wastes are dumped, buried, used for landfill, or incinerated. These actions will further increase the threats to the environment such as polluting the soil, water, and air that negatively influence public health, and also increase greenhouse gas concentration (Kshteri et al., 2018). Human hair consists of 80% keratin protein. Human hair is considered an environmental pollutant and found as municipal waste in the world. Hair waste from barber shops is often accumulated in large amounts as solid waste and it choke and the drainage systems. In rural areas, hair is thrown away in open land where it slowly decomposes over several years. Open dumps of hair generate hair dust, and it gets inhaled in large amounts by the people residing in these areas, which can result in several respiratory problems (Kang et al., 2018; Tamreihao et al., 2019). Thus, keratinized waste is considered biological waste, and it is a huge environmental problem. According to the research, the most effective, dependable, and eco-friendly technologies for degrading keratinized wastes serve as commercial values for numerous applications.

Therefore, this focuses on the current field of research in the methods for the degradation of keratin waste by simple, reliable, and cost-effective way. The latest techniques used in various sectors for the appropriate disposal of keratinized waste were reviewed in this paper along with the degradation of keratin by bacteria, fungus, and microbial enzymes as well as its mechanism of action.

2. Chemical structure of keratin

Keratin is a filamentous protein belonging to the scleroprotein family, produced during the growth process controlled by more than 30 growth factors and cytokinesis (Moll et al., 2008; Kumawat et al., 2018). Keratins are self-arranging fibrous proteins that are present in abundant levels in epithelial and epidermal cells of mammals (Schweizer et al., 2006; Staron et al., 2011). They require special hydrolyzing enzymes as they are insoluble in both hot and cold water. The majority of keratin derivatives are obtained from humans or wool. (Coulombe, & Omary 2002) Keratin can be found in nails, cuticles, claws, hair, wool, feathers, horns, and other extrinsic layers of living beings. The cytoplasm of most epithelial and epidermal cells has bulk quantities of tonofilaments (i.e., keratin) because they protect these cells from any external physical and non-mechanical stresses that result in subsequent cell death (Wang et al., 2016). Keratins provide mechanical strength and support to cells, also undergo reactions with each other and various other classes of linked proteins during their phase of post-translational modification, evolving as a complete

entity (Ni and Dumont 2017). Nearly 54 different functional genes of keratin have been identified in human beings that are regarded as the highest among any other living organism. The ionic, hydrogen, and covalent bond formation abilities of keratin depend on its water susceptibility, solubility, substrate adhesion, and other mechanical properties (Navone and Speight 2018). Keratin is held along with each other by cross-linked disulfide bond. The presence of certain reactive amino acid groups results in the formation of specific and directional bonds with one another. Around 46% of keratin peptides are made up of polar amino acids with the primary contribution of sulfur-containing amino acids like cysteine or cystine. Cysteine are nucleophilic amino acids belonging to the thiol group and they have a strong tendency to reduce the disulfide bonds in keratins since cysteine groups can be easily oxidized. Keratin also contains trace amounts of methionine, lysine, and tryptophan in its structure along with sufficient quantity of amino acids like glycine, alanine, serine, and valine. (Staron et al., 2011).

Based upon their conformations, keratin can be classified into two types, Alpha (α) keratin and Beta (β) keratin (Figure 2). α-keratin is prevalent in invertebrates, and β-keratin is familiar in reptiles and birds and the α and β-keratin levels vary according to the keratinaceous materials. (Hanukoglu et al., 2013)

α-keratins, with covalent disulfide bonds in the matrix, is a material dominated by hydrogen bonds. It is rich in hydrophobic amino acids and is renowned for its flexible yet phenomenal mechanical properties. The α-helix in α-keratin is also known for its resistance to microbial degradation. Keratinocytes, the outermost layer of the epidermis, are synthesized by the epidermal cells. Based on the sulfur content levels, the α-keratin is divided into soft keratin and hard keratin. Hard keratin contains high sulfur content and has a bi-phasic structure which makes them difficult to degrade and it is basically found in bird feathers. On the other hand, soft keratin is found commonly in epithelial cells and can be easily degraded, since they have a low content of disulfide bonds (Vandebergh and Bossuyt 2012).

β-Keratin is rich in cysteine and can instantaneously form disulfide bonds, showing high rigidity and resistance towards degradation. The secondary structure of this protein has α-helix bounded with β-sheet and this forms the body which illustrates as a characteristic filament-matrix structure: 7 nm diameter intermediate filaments for α-keratin, and 3 nm diameter filaments for β-keratin embedded in an amorphous keratin matrix. It makes both the forms of keratin intractable and non-degradable. But these keratin polymers can be degraded by a specific form of protease enzyme called keratinases (Greenwold and Sawyer 2013). The stiffness of H-bond dominated materials is proportional to the density of effective H-bonds. Water can disrupt these structural H-bonds to reduce the hardness of effective bonding, thereby reducing the stiffness of the material (Kumawat et al., 2018; Lee et al., 2012).

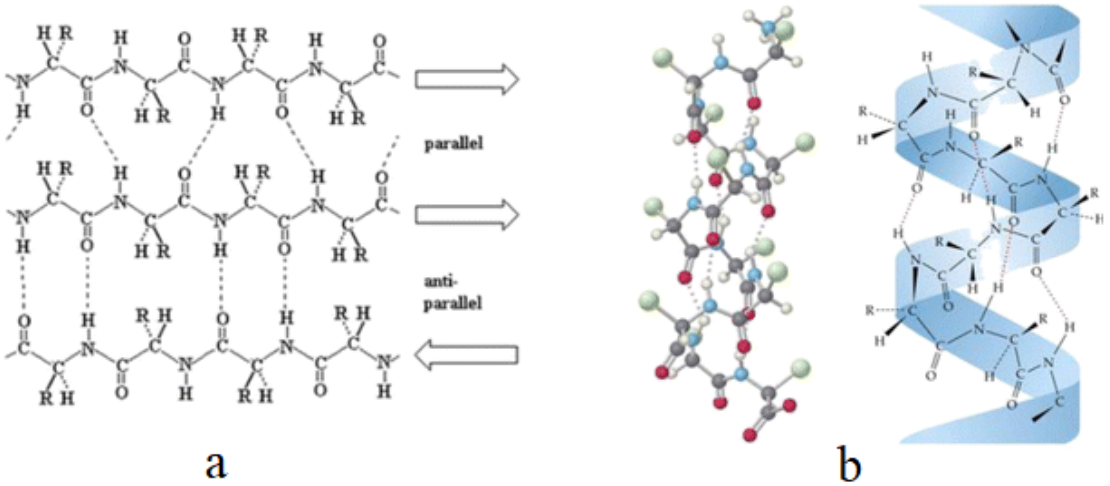

Figure 2. Chemical structural of (a) β and α keratin (Giuffrida, et al., 2018).

3. Prominence of keratin degradation

The disposal of keratin waste products is an important issue due to the high supercoiled nature of the protein. They are stabilized by a cross-linking or hydrophobic interaction of both α & β keratin through disulfide bonds. In the current status, keratinized waste compounds are disposed of effectively by incineration process, where the infectious agents can also be destroyed. On the other hand, these keratinized substances are also disposed of by landfills and burial methods of waste disposal (Tesfaye et al., 2017). Although various methods are employed for the disposal, a high amount of untreated keratinized waste is released into the environment. Thus, it becomes a threat to the environment and public health because of its non-degradable nature (Brandelli et al., 2015) and releases various toxic compounds such as nitrous oxide, ammonia, and hydrogen sulfide (Tamreihao et al., 2019).

Keratin is a high-molecular-weight biological material that is a widespread application in numerous industries (Barone et al., 2005). Keratins from human resources are biocompatible and they can be used in wound healing, biomedical devices, and bone regeneration (Dias et al., 2010a; de Guzman et al., 2013; Wang et al., 2017). Keratins from feathers are the chief source of amino acids (cysteine, glutamine, proline, and serine amino acids) (Table 1) and minerals such as nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, zinc, and copper, which makes them serve as principal energy and mineral sources in animal feeds (Hill et al., 2010; Tamreihao et al., 2019; Onifade et al., 1998).

Even though it has wide applications, keratins derived from various keratin waste are not used efficiently due to the presence of other by-products such as poultry waste, bloodstains, flesh, and fat (Tesfaye et al., 2017). The storage of keratin waste from industries requires a lot of space and temperature, and there is a chance that harmful bacteria, yeast, fungus, and viruses may proliferate, causing a health risk. Despite this, the processed keratinized waste has a useful application that necessitates the most dependable, environmentally friendly, and efficient technology.

Table 1. Predominant amino acids present in the various keratin sources

S. no	Source	Percentage of protein (%)	Predominant amino acids in its structure	Author
1	Feathers	95-98	Glycine, alanine, serine, cyteine and valine	Kumawat et al., 2018
2	Wool	95	Leucin, prolin, valine, glycine, glutamate and cyteine	Gong H et.al., 2016
3	Mammalian hair	80-85	Cysteine, prolin, glycine-tyrosine, glutamate and serine	Imran K et.al., 2014
4	Hoof horn	65-70	Histidine, glycine and tyrosine	Rippon JA et.al., 2013

4. Keratin degradation

The keratin-rich wastes such as feathers, skins, hair, horns showed resistance to degradation due to the covalent disulfide cross-linking in the keratin structure. As a result, several waste management approaches, such as physical, chemical, and biological procedures, have been used to treat keratin-containing wastes from various resources.

4.1. Physical methods of keratin degradation

Incineration is the process of combusting at the temperature of about 850°C, where keratin waste and potential infectious agents get converted to CO_2 and water (Dube et al., 2014). Since this procedure requires a high temperature, it has a significant operating and maintenance cost (Sharma and Gupta. 2016) and it also emits pollutants into the environment, resulting in unpleasant smells and toxic runoff that harms nearby livestock and ecosystems (Gupta A, 2014). Landfilling is the most widespread method of organized waste disposal in several places around the world (Remigios. 2010) is also a disposal method of keratin wastes used traditionally all these years (Onifade et al., 1998; Mehta et al., 2014). But improper

discarding of keratin wastes by landfilling leads to the transmission of diseases (Tronina and Bubel. 2008) and landfill leachate destroys the ecosystem and greenhouse gases (Vuppu et al., 2012). Even though landfilling is the most cost-effective technique for disposing of keratin waste, it is inefficient. Composting is another aerobic biological process degrading organic material and recycling feather waste which reduces the harmful pathogens present along with keratin and compost products used as manure (Davalos et al., 2002; Thyagarajan et al., 2013; Liu et al., 2016). Another alternative method for the disposal of keratin waste is by mechanical grinding into valuable products. It is a sequence of three steps that includes autoclaving (hydrolyzing), grinding, and drying (Jaouadi et al., 2013). This ground powder can be used as animal feed (nitrogen source) or as manure (Hadas and Kautsky. 1994). Even though it has a wide range of uses, it has certain drawbacks, such as the need for high temperatures and the loss of heat-labile amino acids while grinding (Wang and Parsons. 1997; Latshaw et al., 1994). Because of the large number of hazardous gases produced, disposal of keratin waste by incineration, landfilling, composting, and mechanical grinding is limited (Verheyen et al., 1996).

4.2. Chemical methods of keratin degradation

4.2.1. Hydrolysis process

One of the traditional methods of keratin waste disposal is by chemical hydrolysis (acid, base, catalyst) that requires certain conditions such as high temperature and pressure. Even though it is a slow and highly efficient process, the loss of some essential amino acids leads to a low nutritional product. The chemical process increases the production of certain gases like CO, SO_2 into the environment and causes respiratory diseases, cardiovascular diseases, and cancer, among other illnesses (Gupta and Ramnani. 2006).

4.2.2. Alkaline method

The non-degradability nature of keratin structure is mainly due to disulfide bonds. Hence, reducing the disulfide linkage with thiol chemicals destabilizes the disulfide bonds in the polypeptide chain at various processing conditions (Amin shavandi et al., 2017). Traditionally, wool is solubilized by strong, and hot alkali solutions and this process has been known for many years. In an alkali solution, hydrogen forms of sulfate and carboxylic groups are dissociated, causing sulfur-containing amino acids, such as cysteine residue, to degrade. But in large-scale the process requires a large volume of alkali solution for dissociation and a large amount of acid reagent to precipitate the protein and that produces an unpleasant odor (Amin shavandi et al., 2017). Thus, considering all the disadvantages of the chemical and physical methods, the management of keratin waste needs an alternative process with an environmentally friendly and more reliable efficient technique. Thus, the drawbacks of the proposed factors, the use of microbes to control keratin waste looks to be a feasible alternative, motivating scientists to do study in this area.

4.3. Biological Method

Because of high contamination consequence, energy, and cost of the physical and chemical methods, the biological method of keratin waste degradation is the better alternative. This is a cost-effective, highly efficient, eco-friendly method which produces toxin free by-product (Kornillowicz et al., 2011). Keratin wastes can be decomposed with the aid of certain microorganisms which are naturally present in the soil called keratinophilic microflora. This group represents microorganisms such as bacteria, fungi, and actinomycetes that are capable of degrading the highly stable recalcitrant keratin protein (Tarun et al., 2018; Singh and Kushwaha. 2015; Sinoy et al. 2011). This keratinophilic microflora decomposes the stubborn keratin protein via enzymatic digestion by keratinolytic enzyme called keratinase. This keratinase enzyme is a protease that possesses a peculiar ability to degrade keratinous waste into beneficial amino acids that can be used as a nutrient supplement in animal feed (Wan-Ling Wu et al., 2017). The keratinolytic activity of the keratinase enzyme and the microorganisms that produce them have increasing industrial applications and have attracted wide biotechnological interest. The amino acid that is obtained as the by-product has a wide range of applications, for instance, it is utilized in the cosmetic industry.

5. Microbial keratin degradation

Microbial keratin degradation involves various steps such as adhesion, colonization, keratinase production, and deprivation of the substrate (Suzuki et al., 2006). The microbial breakdown of keratin waste starts with

colonizing non-keratinous components like lipids before moving on to keratin degradation (Marchisio. 2000). The keratinolytic microorganisms use keratin as a nutrient source for their growth and keratinase enzyme is produced by bacterium species and fungal species as an extracellular protease.

5.1. Bacteria

Many bacterium species produce the keratinolytic enzyme keratinase for the degradation of keratinous waste. The Bacillus sp. and Chryseobacterium sp. are characterized as efficient decomposers of keratin (Lange et al., 2016). Bacteria can multiply very fast, and the enzymes they produce can sustain their activity under various conditions. Certain enzymes produced by bacterium species can be active at high temperatures and different pH levels (Qingxin Li. 2019). It is stated that the thermophilic bacterium, Meiothermus taiwanese also has a greater potency to degrade the chicken feathers (Wan-Ling Wu et al. 2017). Other bacteria capable of generating keratinolytic enzymes include Pseudomonas, Stenotrophomonas, Brevibacillus, Fusarium, Xanthomonas, Nesterenkonia, Serratia, and Geobacillus. The keratinase produced by bacteria breaks the disulfide cross-links into free thiol groups with the protonation of an amino group and other groups in keratin, thus creating a positive charge on its surface. As a result, the keratin becomes a pseudo cationic biopolymer. Hence this the end product can be used as a valuable raw material for industries like pharmaceuticals and cosmetics (Swati Sharma and Arun Gupta. 2016). The protease enzyme generated by this bacteria species can degrade keratin as well as other proteinaceous substrates. This ability of the protease enzyme has a high commercial value in the food processing industry, detergent industry, and leather industry (Gupta et al., 2013).

5.2. Fungi

Many fungal species have high keratinolytic activity, and these include the following genera: Aspergillus, Trichurus, Stachybotrys, Gleomastis, Chrysosporium, Cladosporium, Monodictys, Alternaria, Curvularia, Myrothecium, Paecilomyces, Fusarium, Urocladium, Geomyces, Sepedonium, and Doratomyces (Marcondes et al., 2008). A feasible microbial technology for obtaining keratinolytic enzymes is laboring the keratinophilic fungi that utilize the keratinous waste as a fermentation substrate. The fungal species can be grown on agar medium plates containing agar-15, $MgSO_4.H_2O$-0.5, KH_2PO_4-0.1, $FeSO_4.7H_2O$-0.01, and $ZnSO_4.7H_2O$-0.005 and also with 1% feather powder as a source of nitrogen & carbon (Wawrzkiewicz et al., 1991). In submerged conditions with a medium containing porcine nail as the source of carbon and nitrogen, it shows that only some isolates of Fusarium, Acremonium, and Geotrichum exhibited to be the potent producers of extracellular keratinase (Friedrich et al., 1999). The potency of filamentous fungi is a result of a combination of keratinase, mechanical keratolysis (mycelial pressure on the keratinous substrate), sulfitolysis (breakdown of disulfide bonds by the sulfite excreted by mycelia), and proteolysis (cleavage of protein bonds) (Gupta and Ramnani, 2006). The majority of identified fungal keratinases belong to the S8 family of serine proteases, exemplified by proteinase K-like proteases found in Paecilomyces marquandii and Doratomyces microsporus Fungal strains of D. microsporus and P. marquandii were grown through submerged fermentation, and the culture filtrates exhibited keratinolytic activity after 40 hours for both strains. The enzymatic activity increased steadily until it reached its peak, reaching 49.5 U/ml after 95 hours for D. microsporus and 230.6 U/ml after 110 hours for P. marquandii. Subsequently, the culture filtrates were collected, concentrated, dialyzed, and lyophilized, resulting in the extraction of 0.25 g of crude keratinase from D. microsporus and 0.4 g from P. marquandii per liter of fermentation broth. Purification procedures, as outlined in the Materials and Methods section, led to a 3.8-fold increase in specific activity to 1,005 U/mg for D. microsporus and a 4.9-fold increase to 326 U/mg for P. marquandii. SDS-PAGE analysis revealed a distinct single protein band for each purified keratinase. (Gradisar et al. 2005). The keratinase enzymes produced by the fungal species can be altered by the addition of nutrient sources. For instance, in some cases, the addition of carbon sources like starch and glucose highly inhibits the keratinase production of Streptomyces sp. (Mabrouk. 2008). However, certain fungal species may generate keratinase even in the absence of a carbon source by absorbing keratin as a carbon and nitrogen source (Gousterova et al., 2005). For obtaining the maximum yield of keratinophilic fungi and sporulation the optimum conditions such as the temperature ranging

from 15°- 35°C and pH is around 5.0 – 8.0 should be maintained (Tarun et al., 2018). High keratinase activity can attain when the temperature ranges from 25°- 40°C and the pH is around 7.5 – 9.0 (Saber et al., 2010). High keratinase activity can attain when the temperature ranges from 45°C -65°C and the pH is around 6.0-11.0 (Gradisar et al. 2005).

6. Mechanism of keratinases on keratin degradation

The characteristics such as molecular weight, temperature, amino acid sequence are different in keratinases produced by bacteria and fungi (Table 2). Keratinases are enzymes that can break down the refractory and insoluble keratins found in hair, feathers, and wool (Qingxin Li 2019). The mechanism of keratin degradation by keratinases involves the enzymatic cleavage of the proteinaceous structure of keratin. Keratin is a fibrous protein found in various natural materials such as hair, feathers, wool, and nails, and it is characterized by its tough and insoluble nature. Much of the research shows that keratinase does not break the disulfide bonds and it requires additional steps to degrade the keratin. Also certain studies shows that only crude enzymes have a higher keratin degradation rate when compared to the purified enzyme and minimum two enzymes are responsible for keratin degradation. The process of keratin degradation involves two main reactions (i) sulfitolysis (release of sulfide favors the breakdown of keratin's disulfide bonds) and proteolysis (lysis of protein) by keratinases produced by keratinolytic microbes (Gupta and Ramnani. 2006; Riffel et al., 2003; Brandelli et al., 2010). Sulfitolysis is the vital process of keratolysis (Blyskal. 2009) as disulfide bonds are removed by the enzymes sulfide reductases or reducing agents. With the help of one enzyme, keratin with cysteine residues can be produced in the reduced form thus, the cleavage sites is exposed to protease. In this process, microorganisms discharge sulfide, which is accountable for the breakdown of keratin's disulfide bonds (Sharma and Rajak. 2003). In proteolysis, degradation of keratinous substrates is proficient due to their ability to secrete extracellular keratinase enzyme into the medium (Lange et al., 2016; Singh and Kushwaha. 2015). In general different mechanisms are carried out in keratin degradation by bacteria and fungi to cleave the polyproteins (Qingxin. 2019). The bacterial and fungal keratinases shows the difference in their physical and chemical characteristics such as amino acid sequence, molecular weight, optimal pH, and temperature toward keratins from different origins (Brandelli. 2008).

Table 2. List of some bacteria and fungi degrading keratin and its keratinase activity

S.No	Strain	Substrate	Keratinase activity	References
BACTERIA				
1.	Xanthomonas sp.	Feather	69.0 U/mL	Jeong et al., 2010
2.	Chryseobacterium sp.	Feather	967 U/mg	Riffel et al., 2007
3.	Bacillus sp.	Feather	256 U/mL	Liu et al., 2013
4.	Serratia sp.	Milk	130 U/mL	Khardenavis et al., 2009
5.	Pseudomonas sp.	Feather	23.7 U/mL	Dhiva S et al., 2020
6.	Stenotrophomonas sp.	Feather	32.0 U/mL	Zhang et al., 2009
FUNGI				
1.	Chrysosporium sp.	Feather	10.51 KU/mL	Jaroslova et al., 2014
2.	Aspergillus sp.	Feather	113.50 U/mL	Karina et al., 2020
3.	Sterptomyces sp.	Casein	265 U/mg	Tatineni R et al., 2008
4.	Trichophyton sp.	Feather	5 KU/mL	Anbu et al., 2005
5.	Fusarium sp.	Feather	243.25 U/mL	Karina et al., 2020
6.	Paecilomyces marquandii	Crude Keratinase	326 U/mg	Gradisar et al. 2005
7.	Doratomyces microsporus	Crude Keratinase	1005 U/mg	Gradisar et al. 2005

Most researchers isolated and described the pure form of the enzyme utilizing keratin-derived substrates such as azokeratin, keratin azure, human hair, bovine horn, feather, and keratin powder (Gupta and Ramnani, 2006). Keratinases from bacteria and fungi showed activity at temperatures ranging from 28 to 90°C under optimum enzymatic conditions, including buffer pH. At pH values ranging from 5 to 13, the enzymes were able to sustain their activity. (Gupta and Ramnani, 2006; Brandelli et al., 2015). Studies have also found that enzymes from fungus, bacteria and other extremophiles have greater optimum temperatures, resulting in increased keratin breakdown efficiency (Kanoksilapatham and Intagun, 2017). The P. marquandii keratinase is distinctive for its temperature optimum at 60 to 65°Cat optimum pH 8, whereas most other reported keratinases, except for thermostable ones, are active only up to 50°C. (Gradisar et al., 2005). Thermal stability is enhanced by the existence of disulfide links in the protease, stability (Wu et al., 2017). Recombinant methods are used to over-express and purify heat-stable keratin from Meiothermus taiwanensis WR-220 (Wu et al., 2017). For a complete characterization of keratinases, including evaluation of their P1 sites, a keratinolytic assay, biochemical and structural study is required.

7. Recombinant approaches for keratin degradation

The ideology to bring about modifications in the mechanism of keratin degradation has encountered a lot of approaches through recombinant strategies in recent years. Due to their unique physical and chemical characteristics, keratin and keratin-based materials cannot be degraded at ease. Keratinases, these proteolytic enzymes are considered to be a potential candidate to degrade keratin, due to their ability to hydrolyze keratin polymers. To ameliorate the reactivity and structural properties of keratinases, its purified form is obtained from various microbes (i.e bacteria, fungi, yeast) and are re-engineered from their native form. The recombinant strain usage to degrade keratin and keratin-based materials was performed initially by (Riessen, S., & Antranikian, G. (2001)) on 2KXI isolate, considered to be a new species from the thermoanaerobacter genus. The strain possessed the capability by virtue to degrade native keratin using it's extracellular and intracellular proteases, which showed peak activity at pH 7 and 8 and at temperatures 60℃ and 80℃ respectively. Following this strain (Yong et al. 2013) and (Yong et al. 2020) performed an in-silico analysis on Bacillus subtilis S1-4, that primarily focused on B. subtilis WB600 and Escherichia coli DH5α. For cloning, E. coli DH5α has been used for recombinant expression of foreign genes, and an extracellular protease deficient strain B. subtilis WB600 has been used. This BsKER71 has the ability to work at a variety of pH levels and temperatures to hydrolyze various proteases. In search for a novel yet a thermostable microbe for degrading keratin (Nam et al., 2002), performed meticulous analysis on Fervidobacterium species. The F. islandicum AW-1 produced keratinolytic protease that showed exceptional activity at a pH of 9 and temperature 100°C on the course of degrading/solubilizing keratin. To obtain comprehensive results, (Kang, E et al., 2020) and his team had performed RT-PCR and q-PCR on the enzymes and employed recombinant proteases on biochemical assays to extrapolate information on enzyme responses for keratin degradation. Consequently, (Jin H et al., 2017), over-expressed Gallus gallus chromosomes 2 and 27 β-keratin-encoding genes in Escherichia coli. Further qualitative analysis using this gene revealed protease K activity that exhibited keratinolytic activity for the recombinant keratin. On a more conscientious scale, (Saeid Alinezhad and Amir Mirabdollah) performed PCR amplification, and cloned the keratinase (kerA) gene of Bacillus licheniformis into Bacillus megaterium expression vector. After 24 hours of incubation with recombinant B.megaterium his team observed macroscopic digestion of feathers. (Radha, S., & Gunasekaran, P. (2009)) also had performed a similar experiment where the expression of the cloned keratinase gene from Bacillus licheniformis MKU3 occurred in Bacillus megaterium MS941 as well as in Pichia pastoris X33 which produced increased folds of keratinase. Glycosylation offered by the yeast system ameliorated the ability of keratinase to degrade keratin substrates. (Dong, Y. Z et al., 2017) analysed a new resultant KerP-expressing strain PT5(DMT-KerP) by integrating the plasmid pDMT into B. subtilis PT5 chromosome.The PCR amplification of KerP gene along with primers KP01 and KP02, were cloned with the integrant plasmid pDMT to generate pDMT-KerP, using NruI and BamHI. This recombinant strain, after 7 days of incubation in a feather defined medium, had completely digested the feathers where the KerP gene showed an optimal activity at 60 °C and at a pH of

9. (Wang, L et al., 2019) worked on the extracellular protease keratinolytic protease (KERTYT) secreted by Thermoactinomyces sp. YT06. The strain's tendency to degrade feathers was analysed and The kerT1 gene (1170 bp) encoding keratinase was cloned and expressed in Escherichia coli BL21(DE3). The strain rKERTYT showed an optimal activity at a temperature of 65 °C and at a pH of 8.5 and the protein remained stable from 50 to 60 °C and pH 6–11. (El-Bondkly, A. M., & El-Gendy, M. M. (2010)) performed by protoplast fusion and genetic recombination between 2 strains ESRAA1997 and ALAA2000, lead to their discovery of a potent keratinolytic fusant Micromonospora strain AYA2000 which had an optimal activity at 60°C. Further advances in the field of keratin degradation employing keratolytic enzymes is backed by recombinant technology to incorporate some of the promising microbial strains to pave the way for a sustainable environment.

8. CONCLUSION

Keratinase is a kind of extracellular proteolytic inducible enzyme that may degrade keratin substrates that are insoluble. Keratinase enzymes are distinct from other proteases as they breakdown compact keratin components. Understanding the nature of keratinases' effective hydrolysis of keratin is thus crucial for both industry and the environment. When propagated on keratinous substrates, microbial keratinases are mostly extracellular; however, cell-bound (Friedrich and Antranikian. 1996; Onifade et al., 1998; Rissen and Antranikian. 2001; Nam et al., 2002). The isolation of keratinases from non-pathogenic bacteria and their capacity to break down keratin into economically valuable keratin products has boosted their relevance (Onifade et al., 1998) Therefore, microbial degradation favors the processing of keratin waste without pretreatment which makes this a simple and cost-effective method.

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