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Research Article

MICROBIAL DEGRADATION OF KERATIN AND IT'S AGRO-INDUSTRIAL PROSPECTIVE APPLICATIONS

Bhavani Gandhe[1]., Kavitha Anguluri[2]., Shrey Bodhankar[3] and Sai Shiva Krishna Prasad Vurukonda[4*]

[1]Department of Biotechnology, Osmania University, Hyderabad, Telangana State, 500007, India
[2]Department of Microbiology, St. Francis College for Women, Hyderabad, Telangana State, 500016, India
[3]Central Research Institute for Dryland Agriculture, Hyderabad, Telangana State, 500059, India
[4]Department of Life Sciences, University of Modena and Reggio Emilia, via Amendola 2, Modena, Italy

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A B S T R A C T

Keratin is ubiquitous and belongs to the family of fibrous structural proteins found in nature. It is abundant in mammals, reptiles and birds and fishes and in mostly all parts of the body, including horns, hooves, fur, wool, quill, skin, feathers, slime and beaks. Keratinases are stimulating proteolytic enzymes which are capable to degrade the insoluble protein keratin. These enzymes are produced by diverse microorganisms, microbial keratinases have become potentially important in agro-industries due to their target for the hydrolysis of highly rigid, strongly cross-linked structural polypeptide "keratin" recalcitrant to the commonly known proteolytic enzymes trypsin, pepsin and papain. The microbial degradation of keratin wastes is considered as a potential biotechnological alternative for recycling and valorization through keratinolytic beneficial microorganisms. In context to this, the present review is focused on the microbial degradation of keratin and its ecology, mechanism of action and various applications.

INTRODUCTION

Keratin is a fibrous and complex structural protein and is the most abundant polymer in nature next to cellulose and chitin. A wide group of animals (mammals, fish, birds, and reptiles) has developed diversified keratin as a structural part of their outer body protection and is a abundantly present in skin, hair, feather, horns, hooves, cloves, nails, beaks, reptilian osteoderm, and fish teeth and slime (McKittrick *et al*., 2012). Keratin is an insoluble macromolecule requiring the secretion of extra cellular enzymes for biodegradation to occur and it's comprises long polypeptide chains, which are resistant to the activity of non-substrate-specific proteases. Adjacent chains are linked by disulphide bonds thought responsible for the stability and resistance to degradation of keratin (Safranek and Goos, 1982). The degradation of keratinous material is potentially important both medically and biotechnologically (Shih, 1993; Matsumoto, 1996). World-wide poultry processing plants produce millions of tons of feathers as a waste product annually (Santos *et al*., 1996), which consists of approximately 90% keratin and is largely responsible for their high degree of recalcitrance. The keratinous wastes are increasingly accumulating in the environment mainly in the form of feathers, hair, horns, hooves and nails generated from various industries. The sewage and bottom sediments of rivers and canals contains an enormous amount of hidden keratinous waste because of daily shaving habits in metros. Today, it is also becoming a part of solid waste management and it is difficult to degrade and recycling of such wastes is increasing attention (Singh and Kushwaha, 2015).

The enzyme keratinase is used to designate the subset of proteases which have keratinolytic activity. The more we study the enzymatic decomposition of keratin, the more obvious it becomes that a distinction between true keratinases and other proteases is not straightforward. Recent findings suggest that several proteases may have keratinolytic activity but such activity only leads to full keratin decomposition if several different keratinolytic enzymes act together (Lange *et al*., 2014; Huang *et al*., 2015a). Most published keratinase reviews by Brandelli (2008); Daroit and Brandelli (2014); Sahni *et al*., (2015) are mainly focused on bacterial keratinases or include only very limited information about fungal keratinases (Onifade *et al*., 1998; Gupta and Ramnani, 2006; Brandelli *et al*., 2010; Korniłłowicz-Kowalska and Bohacz, 2011; Gupta *et al*., 2013; Lange *et al*., 2016). In nature, various beneficial microbes work together in breaking down the protein rich keratin structures and is abundantly present as nitrogen source. The present review focused on mechanism and role of microbial enzymes, which are examined from the perspective of a synergy between

Corresponding author:* **Sai Shiva Krishna Prasad Vurukonda
Department of Life Sciences, University of Modena and Reggio Emilia, via Amendola 2, Modena, Italy

biocatalysis and chemical catalysis in keratin decomposition for various purposes.

Ecology of Keratin Degrading Microorganisms

Ecology of microbial species for the degradation of keratin started many years back, keratin-degrading microorganisms are almost ubiquitous in nature, although preferentially (obligatorily or facultatively) thriving on keratinous substrates. Interestingly the best-studied groups are the dermatophytes because of the pathological interest of most investigators (Grappel and Blank, 1972; Higuchi *et al*., 1981; Wawrzkiewicz *et al*., 1987; Apodaca and McKerrow, 1989; Hanel *et al*., 1991; Wawrzkiewicz *et al*., 1991; Porro *et al*., 1997). A vast group of bacteria, actinomycetes and fungi are known to be keratin degraders. Table 1, adapted from Gupta and Ramnani (2006), showing some of the important microbial keratinases. Besides these some of the actinomycetes, in particular *Streptomyces* group, *viz. S. fradiae* (Novel and Nickerson, 1959), *Streptomyces* sp. A11 (Mukhopadhyay and Chandra, 1990), *S. pactum* (Bockle *et al*., 1995), *S. albidoflavus* (Letourneau *et al*., 1998), *S. thermoviolaceus* SD8 (Chitte *et al*., 1999) and *S. graminofaciens* (Szabo *et al*., 2000) and the *Thermoactinomyces* group, *viz*. *T. Candidus* (Ignatova *et al*., 1999) and another *Thermoactinomyces* sp. (Gousterova *et al*., 2005), is commonly described as keratin degraders with an ability to act on a wide variety of keratin substrates including hair, wool and feather. The most keratinolytic group among fungi belongs to fungi *imperfectii* including the following genera: *Chrysosporium*, *Aspergillus*, *Alternaria*, *Trichurus*, *Curvularia*, *Cladosporium*, *Fusarium*, *Geomyces*, *Gleomastis*, *Monodictys*, *Myrothecium, Paecilomyces, Stachybotrys, Urocladium, Scopulariopsis, Sepedonium, Penicillium, Doratomyces*. However, they do not have much commercial value as most of them are categorized as dermatophytes (Gradisar *et al*., 2000). The characteristic of keratinolysis is widely distributed among the microbial world. However, only a few have reached commercial exploitation, for example, from *Bacillus* sp. particularly *B. licheniformis* and *B. subtilis* have been extensively studied due to their effectiveness in terms of feather degradation (Manczinger *et al*., 2003; Thys *et al*., 2004).

Mechanism and mode of action of keratinases

The proper mechanism and mode of action of keratinases on keratin degradation is still not fully understood **(**Fang *et al*., 2013**)**. It is recognized that the reduction of disulfide linkages is required for efficient hydrolysis of native keratinolytic substrates. In this sense, the keratinolysis needs the synergistic action of the proteases with any of the various redox mechanisms, such as reductases, sulfite production, reducing agents, or redox potential of cells (Yamamura *et al*., 2002; Rahayu *et al*., 2012). The reducing agents often used are β-mercaptoethanol and dithiothreitol (DTT). Experiments by Yamamura *et al*., (2002) purified and characterized a disulfide reductase-like protein from *Stenotrophomonas* sp. and revealed its role to degrade keratin with the cooperation of protease D1. The proposed mechanism for extracellular enzymatic keratin degradation by bacteria/fungi comprises two steps: (i) initially, the disulfide bonds in keratin are attacked by disulfide reductase-like enzymes, resulting in the increase of free thiol groups and weakening protein structure (Yamamura *et al*., 2002; Nagal and Jain, 2010) and (ii) keratinase cleaves the peptide bonds of β-pleated sheet and releases different amino acids and peptides (Nagal and Jain, 2010; Gupta and Singh, 2014**)**. Efficiency of keratinase in keratin degradation is totally depends on its source, culture conditions, chemical composition and nature of substrate (Mazotto *et al*., 2011). Initial step in the mechanism of keratin degradation exhibited by disulphide reductase by cleaving the disulphide bridges of β-sheet (Laba and Szczekala, 2013). It is also act as a crucial enzyme in the cellular antioxidant system protects cell against free radicals (Tandogan and Ulusu, 2006).

Microbial Degradation of Keratin and Its Applications

Microbial keratinases are considered as promising enzymes for several purposes, including applications in feed, fertilizer, detergent, leather and also for pharmaceutical and biomedical applications (Onifade *et al*., 1998; Gupta and Ramnani, 2006; Brandelli, 2008). Despite this, the industrial application and hence the market demand of keratinases, as compared to other industrial enzymes, is still beginning stage (Rai *et al*., 2009).

As fertilizers

Plant metabolizing inducer organic fertilizers were prepared by using sulfur bound amino acid solution (Chikura *et al*., 1994). The composting of chicken feather was also used to produce biofertilizers (Ichida *et al*., 2001; Kornillowicz-Kowalska and Bohacz, 2011). Keratin is a good nitrogen source used to prepare the fertilizers (Reddy, 2015). Bacteria and fungi produces keratinolytic enzymes which help to degrade the waste biomass of keratin and both keratinolytic bacteria and fungi are proposed for use in composting. Among bacteria *Bacillus* genus produces plenty of keratinolytic enzymes and actinomycetes also contribute to keratin degradation (Letourneau *et al*., 1998; Lin *et al*., 1992; Ichida *et al*., 2001; Tiquia, 2005). Feathers are rich in nitrogen which makes them to be used as excellent fertilizers but the presence of cystine linkages, makes it difficult to degrade, so it made feather biomass less interesting to be used as a fertilizers (Hadas and Kautsky, 1994; Gurav and Jadhav, 2013). Some studies have been carried out to increase the utility of waste feather as biofertilizer. In a previous study, the release of nitrogen was increased by treatment of feather with *Chryseobacterium* sp. these hydrolyzed feather fertilizers were used for banana plants and can be applicable as root inoculum and foliar spray for other cultivation crops (Gurav and Jadhav, 2013). Feather waste treated with thermophilic actinomycetes strain was also used as a fertilizer for rye grass cultivation **(**Gousterova *et al*., 2012**)**.

In leather industry

Leather processing technology involves a series of operations, amongst which pre-tanning contributes to the major amount of pollution (approximately 70%). Sodium sulfide, lime and solid wastes generated during pre-tanning are mainly responsible for increased biological oxygendem and (BOD), chemical oxygen demand (COD) and total dissolved solids (TDS) (Thanikaivelan *et al*., 2004). Enzymatic leather processing involves use of mixture of enzymes, among which proteases, lipases and carbohydrases are well exploited in various pre-tanning stages (Saravanabhavan *et al*., 2004; Thanikaivelan *et al*., 2004). In addition, keratinolytic proteases lacking collagenolytic and having mild elastolytic activities are increasingly being explored for the dehairing process. They would help in the selective breakdown of keratin tissue in the follicle, thereby pulling out intact hairs without affecting the

tensile strength of leather (Macedo *et al*., 2005). Previous reports indicate that keratinases could be useful depilating agents are available (Letourneau *et al*., 1998; Bressollier *et al*., 1999; Allpress *et al*., 2002; Friedrich and Kern, 2003). According to Macedo *et al*., (2005), keratinase from *B. subtilis* S14 was reported to eliminatethe need for toxic sodium sulfide. Thus, sulfide-based "hair-destroying dehairing" processes that pose an environmental threat by increasing the BOD could be replaced by keratinase-based cleaner "hair-saving dehairing" technology.

As detergents

Proteolytic enzymes have dominated the detergent market since ancient times. In fact, approximately 89% share of detergent enzymes is captured by alkaline proteases, with Novo Nordisk and Genencor International being the major suppliers (Gupta *et al*., 2002). In fact, there is always a need for new enzymes with prospective properties that can further widen the scope of enzyme-based detergents.

Table 1 List of microbial keratinases

Microorganism	Source of isolation	Keratinolytic activity	Reference
Bacillus licheniformis PWD1	Poultry waste	Chicken feather	Williams *et al*., 1990; Lin *et al*., 1992
Fervidobacterium pennavorans	Hot spring	Chicken feather	Friedrich and Antranikian, 1996
Kocuria rosea LBP-3	Soil	Chicken feather	Vidal *et al*., 2000; Bernal *et al*., 2003
Bacillus subtilis KS1	Poultry waste	Chicken feather	Kim *et al*., 2001; Suh and Lee, 2001
Bacillus pumilus, *Bacillus cereus*, *Bacillus* sp. FK 28	Soil	Chicken feather, feather meal	Pissuwan and Suntornsuk, 2001
Thermoanaerobacter keratinophilus sp. nov.	Geothermal hot spring	Chicken feather, wool	Riessen and Antranikian, 2001
Bacillus licheniformis K-508	Rotted feather	Chicken feather	Rozs *et al*., 2001; Manczinger *et al*., 2003
Xanthomonas maltophila POA-1	Poultry waste	Feather meal	De Toni *et al*., 2002
Fervidobacterium islandicum AW-1	Geothermal hot spring	Chicken feather	Nam *et al*., 2002
Stenotrophomonas sp. D1.	Soil containing deer fur	Keratin Powder, chicken feather	Yamamura *et al*., 2002a
Bacillus pseudofirmis AL-89, *Nesternokia* sp. AL-20	Alkaline mud and soil samples	Chicken feather	Gassesse *et al*., 2003
Chryseobacterium sp. kr6	Industrial poultry	Chicken feather	Riffel *et al*., 2003
Bacillus sp. FK 46	Soil	Chicken feather	Suntornsuk and Suntornsuk, 2003
Bacillus licheniformis RG1	Compost	Chicken feather	Ramnani and Gupta, 2004
Microbacterium arborescens kr 10	Industrial poultry wastes	Chicken feather	Thys *et al*., 2004
Bacillus licheniformis, *Bacillus subtilis* ATCC 6633	Soil	Chicken feather	Zerdani *et al*., 2004
Bacillus subtilis S 14	Bovine hair, skin wastes, soil	Bovine hair	Macedo *et al*., 2005

As biomaterials

The extensive studies on keratin led to the development of many keratin-based biomaterials for use in biomedical applications. This foundation is based on several key properties of keratins that contribute to the overall physical, chemical and biological behavior of these biomaterials. First, extracted keratin proteins have an intrinsic ability to self-assemble and polymerize into porous, fibrous scaffolds. The spontaneous self-assembly of keratin solutions has been studied extensively at both the microscale (Steinert and Gullino, 1976; Thomas *et al*., 1986; van, 1987) and macroscale levels (Ikkai and Naito, 2002). This phenomenon of self-assembly is evident in the highly conserved superstructure of the hair fiber and, when processed correctly, is responsible for the reproducible architecture, dimensionality and porosity of keratin-based materials. In addition, keratin biomaterials derived from wool and human hair have been shown to possess cell binding motifs, such as leucine-aspartic acid-valine (LDV) and glutamic acid-aspartic acid-serine (EDS) binding residues, which can support cellular attachment (Tachibana *et al*., 2002; Verma *et al*., 2008). Together, these properties create a favorable three-dimensional matrix that allows for cellular infiltration, attachment and proliferation. Like other intermediate filaments, keratins are also believed to participate in some regulatory functions that mediate cellular behavior (Izawa and Inagaki, 2006; Magin *et al*., 2007). Thus, the conservation of biological activity within regenerated keratin biomaterials could prove advantageous for the control of specific biological functions in a variety of tissue engineering applications (Rouse and Van Dyke, 2010).

Keratinases can bind and hydrolyze solid substrates like feather, which is an important property of detergent enzymes as they are required to act on protein substrates attached to solid surfaces, making them attractive additives for hard-surface cleaners. They could also help in the removal of keratinous soils that are often encountered in the laundry, such as collars of shirts, on which most proteases fail to act (Gassesse *et al*., 2003). An extended application of keratinases in detergents is their use as additives for cleaning up of drains clogged with keratinous wastes (Farag and Hasan, 2004).

As bio-convertors

Bioconversion of keratin-rich materials into amino acids, peptides and soluble proteins by keratinases and/or keratinolytic microorganisms is a potential method to improve the nutritional value of keratinous wastes as feed supplements (Anbu *et al*., 2008; Cai *et al*., 2008a; Cao *et al*., 2008; Ghosh *et al*., 2008; Khardenavis *et al*., 2009; Syed *et al*., 2009; Prakash *et al*., 2009). Feathers are converted to feather meal and utilized as dietary protein supplement for animal feedstuffs on a limited basis due to the poor digestibility and low nutritional value of the products generated by the conventionally employed hydrothermal processes (Onifade *et al*., 1998; Karthikeyan *et al*., 2007). Particularly, the utilization of keratin-degrading microorganisms has a brighter prospect for developing a new and durable biotechnique for the degradation of keratinous residues, especially feathers, than methods with purified keratinolytic proteases (Matsui *et al*., 2009). Since keratin is deficient in some nutritionally essential amino acids such as methionine and phenylalanine, microbial proteins, amino acids and microbial biomass might

contribute to enrichment of protein hydrolysates (Nam *et al*., 2002; Gousterova *et al*., 2005; Grazziotin *et al*., 2006, 2007; Cortezi *et al*., 2008; Mabrouk, 2008; Vasileva-Tonkova *et al*., 2009). For this reason, the screening for suitable non-pathogenic keratinolytic microorganisms should yield an enhanced protein feedstuff that may reduce the use of soybean and fish meal in livestock diets (Bertsch and Coello, 2005; Grazziotin *et al*., 2008).

As microbial fuel cells

Microbial fuel cells (MFC) are considered as promising technologies for green energy. Chicken feathers were hydrolyzed by microorganisms, for examples using *Pseudomonas aeruginosa* strain SDS3 and the effect of various sources on degradation were studied. Degraded feathers were employed as a source of electricity production using a MFC. Extent of feather degradation was dependent on culture conditions and the bacterial strain used. The *Pseudomonas aeruginosa* strain SDS3 could completely degrade 0.1 and 0.5% of feathers in 3 days and 5 days, respectively but, at 1% concentration, only 80% of feathers even after 7 days of degradation. Presence of mineral sources such as carbon and nitrogen affected the activity and production of the enzyme significantly (Chaturvedi and Verma, 2014). Maximum voltage of 141 mV was obtained after 14 days of incubation. The maximum power density was 1,206.8 mW/m2 and maximum current density was 8.6 mA/m2. It was suggested that chicken feathers could be a good substrate for MFC to produce electricity (Chaturvedi and Verma, 2014; Narendra, 2017).

In pharmaceutical industry

In the biomedical, pharmaceutical and cosmetic industries, keratinases might be utilized in the elimination of keratin in acne or psoriasis, elimination of human callus and degradation of keratinized skin, depilation, preparation of vaccine for dermatophytosis therapy and in the increase of ungual drug delivery (Vignardet *et al*., 2001; Friedrich *et al*., 2005; Gradisar *et al*., 2005; Mohorcic *et al*., 2007). Additionally, keratinases might act to remove the scar and regenerate the epithelia, accelerate healing processes and also in the medicine of trauma (Chao *et al*., 2007). The production of bioactive peptides through the degradation of keratinous wastes is another potentially interesting area to be explored (Riessen and Antranikian, 2001; Matsui *et al*., 2009; Brandelli *et al*., 2010).

Future Aspects

Considerable research efforts are still needed in the field of keratinases characterization to resolve the microbial degradation mechanisms of keratin in nature, for instance keratinases originating from non-pathogenic fungal species are to a large extent unexplored. An obvious initial focus is to characterize and compare the keratinases of fungi belonging to the Eurotiales, Onygenales, and Hypocreales (Lange *et al*., 2016). Use of keratinases in modern medicine may prove to have much broader potentials: First, the recent discovery of the potential of keratinases in breaking down and inactivating misfolded prion proteins (Langeveld *et al*., 2003) could prove to be very important for future treatments, especially if the keratinase used in prion research is contributing to understanding prion pathogenicity and to possibly connecting prions with certain types of dementia (Narayan and Dutta, 2005). Secondly, LPMOs have been suggested to be a part of the pathogenesis of important human pathogens, e.g., cholera (Loose *et al*., 2014; Paspaliari *et al*., 2015). Important aspect to be explored more on the use of keratinases in biomass conversion into biofuels may address the increasing concern on energy conservation and recycling.

CONCLUSION

The present review provides deep insight on microbial keratinases and their prospective agro-industrial applications, which have been considered as proteases due to their ability to act on the tough, rigid, insoluble structural protein called keratin. Keratinases are ubiquitous and are much likely to occupy a special status among many other enzymes. Several keratinases have been isolated and characterized, but large scale industrial purification protocols still need to be established to allow the effective utilization of these enzymes at industrial level. The increasing interest and application on keratinolytic microorganisms and the biochemical properties of their keratinases has been robustly increased. From this present review, a better understanding of the microbial degradation of keratin is being evaluated, for the development of products and processes needed to a proper waste management through recycling keratin-rich agro-industrial byproducts.

Conflict of Interest: The authors declare no conflict of interest.

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