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DEGRADATION OF KERATIN SUBSTRATE BY MICROORGANISMS:A REVIEW

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Keratin is an insoluble proteins present inthe epidermis and important composition of feather, hair, wool, nail, hoofand horns.Keratin is important composition and provide strength to the keratinous substrate.In the environment, feather, hair, wool, nail, hoof and horns are considered as waste material and are major causes of environmental pollution.Keratin is also found in products of agro-industrial processing, dairy industry, poultry farms and slaughterhouses.These keratinous waste are structurally stable and very hard to degrade.The keratinous waste can be managed by biological degradation.Microbial keratinase present in some species of bacteria and fungi is best for the degradation of keratin waste in natural condition.The present review paper explains keratinolytic microorganisms and their keratinase for the biological degradation of keratin waste.

Keywords: keratinolytic microorganisms, keratinase, biological degradation and waste material.

Introduction

Keratin waste are the waste produced by leather industry, poultry industry, slaughter houses and hospitals. Because of their resistance to biodegradation, keratinous wastes in the environment pose a significant threat to the ecosystem. Burning, landfilling, heating under pressure and chemical hydrolysis are common ways for their treatment. These techniques consume a lot of energy are not environmentally friendly. The development of biological/green technologies for the breakdown of keratinous wastes has become critical in light of these issues. Microorganisms degrade keratin and produce biotechnologically important products such as keratinolytic enzymes, peptides, and amino acid-rich keratin hydrolysates. This is a cost-effective and simple method that reduces the environmental problem posed by these wastes in an environmentally friendly manner.

Microbial keratin degradation has been widely explored in recent years, as various bacteria, fungi, and actinomycetes are found to be good keratin degraders and are easily isolated from soil and keratin-rich wastes materials.

Degradation by Bacteria:

Most of the keratinolytic bacteria are Gram-positive bacteria, but Gram-negative bacteria are also found to degrade keratin by few strains. The common species of Keratinolytic bacteria include *Bacillus*, *Brevibacillus*, *Chryseobacterium,* *Stenotrophomonas*, *Pseudomonas*, *Keratinibaculum*, *Paenibacillus*, *Meiothermus*, *Rhodococcus*, *Achromobacter*, *Exiguobacterium* and *Aeromonas*[1,2,3,4]. Several researchers have indicated that species of *Bacillus* have high efficiency to degrade keratin namely *B. licheniformis*, *B. pumilus*, *B. cereus*, and *B. subtilis* [5,6,7]. Some new species of keratinolytic fungi are also isolated including *Bacillus safensis* LAU 13 was isolated by Lateef et al. (2015)[8] and *Bacillus cereus* wasisolated by Ahmadpour et al. (2017) [9].

Degradation by Fungi:

Several fungal species contribute enormously in the recycling of recalcitrant keratinous waste. Most of the research papers for keratin degrading capability have registered mostly fungi as important keratinolytic organisms. Keratin degradation capability among the fungi varies due to some factors such as culture conditions, sources of carbon, nitrogen, and energy source. The morphological feature of filamentous fungi facilitates keratin degradation through the firm attachment of mycelia andpenetration of keratin substrates by fungal hyphae. Keratin degradation capability of fungi has been documented in various literature studies. Various species and strains of fungi have been reported for keratinolytic property including species of *Aspergillus, Penicillium, Chrysosporium, Fusarium, Microsporum, Trichophyton* and *Acremonium*, *Aphanoascus, Chaetomium, Penicillium*[10]. Some other species of fungi which were isolated from soil and poultry waste, have also been recorded for keratinolytic activity including species of *Scopulariopsis*, *Myceliophthora*, *Candida, Cladosporium, Metarrhizium, Neurospora, Cunninghamella* and *Westerdikella* [11,12]. Morophology of fungi are suitable for keratin degradation as their mycelium assist in attachment tokeratin substrate. After attachment fungi perforate keratin by their hyphae. The ideal example to understand the mechanism of keratin degration by nonpathogenic fungi is, *Onygena corvine,* which is a very effective keratin degrader[13].

Dermatophytes are a category of keratinolytic fungi that includes certain members of the keratinolytic fungi. These fungi secrete keratinases, which are necessary for their penetration into the body[15]. The addition of starch and maltose to the fermentation medium at the right pH can improve keratinase synthesis and keratin breakdown. Fungi are the most effective at degrading feathers. The degradation of keratin substrate was achieved by producing nutritional medium with C, N, S, and energy sources in a static situation.The degradation of keratin is also achieved by providing optimum environment condition to the keratinolytic fungi such as pH and temperature.

Degradation by Actinomycetes:

Most of the keratin degrading microbes are among bacteria, fungi, and actinomycetes. Actinomycetese are important keratin degraders because they can hydrolyze a wide range of keratin wastes, including feathers, hair, and wool[16]. In some studies, isolation and keratinolytic activity of some actinobacteria like *Actinomadura*and, *Actinoalloteichus* have been reported for hydrolysis of keratin substrates[17,18]. Some members of *Streptomyces*, viz: *S. fradiae* and *S. gulbargensis* have also been reported to show keratinolytic activity[19]. Species of *Nocardiopsis* have been found effective for keratinolytic activity on feather waste[20].

Keratinase from Actinomyces:

The keratinase obtained from the species of actinomyces and Streptomyces was efficient in degradation of different substrates including keratin azure, human hair, cock feathers, and collage as indicated by experiments.Another important keratinase from actinomycetese was reported by Elhoul et al.,(2016)as a novel thermostable keratinasefrom strain of *Actinomadura viridilutea* and these keratinase displayed effective chicken feather hydrolysis within 96 hours [21].

Similarly, Ningthoujam et al., (2016) reported the production of keratinase from*Amycolatopsis* sp. isolated from a limestone habitat, showing promising keratinolytic activity on keratin azure and chicken feather substrates[22].

Mechanism of Keratin Degradation by Microorganisms:

The exact method by which microbes degrade keratin is unknown. According to some studies, keratinolysis is caused by two mechanisms: sulfitolysis, which involves the reduction of disulfide bonds, and proteolysis, which involves the hydrolysis of peptide bonds of keratin.Sulfitolysis requiresthe presence of disulfide reductases enzyme in the microorganism which work in combination with keratinases for the complete degradation of keratin[23].

According to some researches keratinases are more effective keratin degraders when combine with some enzymes produced by microorganismsfor example disulfide reductasesand cysteine dioxygenase [24]. The main composition of keratin structure formed by disulfide bridges and Disulfide reductase and cysteine dioxygenase initiate keratin by breaking the cross-linkages between disulfide bonds which make the availability of peptide bonds for hydrolysis by keratinaseenzyme.

Ramnani et al., (2005) and Rahayu et al., (2012) combined the different enzymes for keratinolysis of feather waste and concluded that keratinolysis by combinedenzymes were higher than single enzymes[23,25]. Microbial keratin degradation process is also dependent on someinternal factors like rate of agitation and sources of carbon, energy, and nitrogen. It is also suggested that keratin may be added as a sole source of carbon, nitrogen and energy for the efficient keratin decomposition. Availability of keratinase enzyme is also achieved by supplementing the media with keratin source which lead to the production of keratinase enzyme by the microorganisms. During keratin degradation process by microorganisms, exo-polysaccharides can also be synthesized which help in adhesion of microorganisms to the surface of keratin substrate enhancing more degradation of keratin[26].

Application of mechanical force with enzymatic lysis has also been suggested for efficient keratin degradation[27]. It can be concluded that for efficientkeratin degradation, purified keratinase enzyme can be applied to keratin source.

Keratin and its structure:

It is important to understand the structure of keratin for degradation of keratin waste in both natural and *in vitro* conditions. Keratin is fibrous protein and found in the outer covering of almost all reptiles, vertebrates and fishes[28]. Keratin exists widely in nature and found as the third most abundant protein after cellulose and chitin[29]. Presence of high cross linking between disulfide bonds, make it a recalcitrant structural protein. Keratin can be divided into soft keratin and hard keratin. In Soft keratin cysteine content is less than 10% and found epidermis of skin and feathers.Hard keratin has cysteine content of10–14% and present in in hair, nails, wool, claws, and hooves[30].

Keratin is insoluble in water and organic solvent because it forms recalcitrant polymers. Keratin is composed by some forces including disulfide bonds, hydrogen bonds and hydrophobic interactions.This recalcitrant polymer is resistant to degradation by enzymes such as pepsin and trypsin[28].

On the basis of secondary structure, keratins are classified into α-keratin and β-keratin. The classification is based on the presence of α-helix and β–sheet in the secondary structure of keratin. β-Keratin is composed of domains, rich in β-pleated sheets and formcrosslinking via disulfide bonds [31]. Beta-keratin is present in reptiles and birds[32]. The molecular weight of a keratin protein usually in the range of 10–14 kDa[33]. Beta (β) keratin has high cysteine content with disulfide bonds which provide it properties of rigidityand resistance to degradation. Total β-keratin composition of a mature feather is about 80–90% of its content.

α-Keratin consists of α-helical-coil and can form intermediate filaments through self-assembling[34]. Alpha keratins are good in elasticity, toughness, strength and flexibility. These properties of Alpha (α) keratin is due to presence of some amino acid like methionine, phenylalanine, valine, isoleucine and alanine[35]. On the basis of sulfur content, Alpha keratin is further classified into hard and soft keratins[36].

The α-keratin has two keratin polypeptides in its structure, with head to tail structure, in a dimeric coiled coil. Thisself-assembled dimeric structure make head to tail polypeptides. The Dimers again make thier self-assembly to form the tetramers. Four such tetramer units assemble to make an intermediate filament[34]. This type of intermediate filament is found in skin and hair with similar pattern. The uncoiled head is rich in threonine and serine and then make a secondary structure due to phosphorylation and glycosylation activity. N-acetyl glucosamine is found as sugar moiety head domain of the polypeptide. Some molecular techniques can be adopted to increase the stability of keratin such as site specific phosphorylation and Posttranslational modifications including phosphorylation, sumoylation and glycosylation[37].

Different organs may have varied content of α-Keratin and β-keratin. For example, wool has α-Keratinwhile feather is reported to have both keratins[38]. Feathers are found to have 41–67% content ofα-keratins and 33–38% content of β- keratin. Similarly, some other keratin substrate such as hair, bristle and woolhave 50–60% α-keratins, some keratin-associated matrix proteins (20–30 %) and β-keratins. Not only organ but also part of organs may also have different content of keratin protein. The feather's outer rachis contains β-keratin which make it more stable[39].

Sources of keratin protein:

Keratin protein is present inliving organism and these living organisms are largest source for the isolation of keratin protein after their death. Various keratin sources are body parts of living organisms such as feathers, nails, wool, horns, hair, hoof, scales and stratum corneum[40].

Feathers of birds are largest sources of keratin protein andare found mostly as waste products. Feathers are reported to contain 90% of keratin protein[41]. The chicken feathers also have about 90% of keratin making it very stable from water, heat, rain and cold. After slaughtering, feathers are not used and considered as biological waste for the environment[42].

Hair is a good source of keratin and can be taken from human and animals. Animal hairs have more keratin on their hair. Keratin is responsible for the strength and flexibility of the hair.

The human hair contains approximate 80% of keratin protein in its composition. The accumulation of hair after hair cut and animal death causes unusual accumulation of hair, which are counted in the environmental waste.

Human nail is composed of a highlycross-linked keratin network, with several disulfide linkagesand high sulfur content (3.8%). These structural features make human nails a highly strong keratin substrate. Similar conformation with more sulfur content and crosslinking, is found in animal horns making it very tough organ. Some free amino acids, calcium aluminium, copper, iron, zinc, manganese, zinc and chromium also provide toughness to the animal horn[43]. The hoof of animals has high thermal stability due to presence of both α-helical conformation and β-sheet[44]. The extrernal shell of hard keratin is found in beak of birds which make it very stable. So it is clear that different organs are good source of keratin and can be isolated from these body parts.

Keratinases:

The term keratinase is used for important proteases which possess keratinolytic activities. These keratinase proteases show proteolytic activity against stable protein-keratin. Keratinase contain serine or metal protease activity for keratin degradation. Keratinase complete their keratinolytic activity with combined action of some other proteolytic enzymes[16].

Keratinase are enzymes which are synthesized by microorganisms when keratin substrate is available in the media. The keratinases produced by various types of bacteria and fungi of different sources differ in terms of amino acid sequence, molecular weight, optimum pH and temperature[45,46]. Due to keratinolytic activity of keratinase for degradation offeathers, hair and wool, keratinase are really important for industrial applications[47, 48].

Keratinase have important role in different industrial and agrircultural purposes including fertilizers, leather industries, biomedical fields, detergents, cosmetics and material production of Nitrogen fertilizer, biofertilizer, textile industry, pharmaceutical industry and Nano-biotechnology [49]. Keratinase are also being used in poultry industries for improved meat production in broiler chicken[50].

Several researchers have isolated and purified keratinase from microorganisms and characterized the purified keratinase. Keratinase enzyme is produced by supplementing the media with keratin substrates like keratin azure, azokeratin, human hair, cow horn, feather and keratin powder. Microorganisms produce keratinase enzymes when optimum conditions are present including pH, temperature and buffer. Bacteria and fungi produce keratin when suitable temperature range is present ranging from 28-40^{0}C and mostly between 28-35^{0}C. Similarly, these microorganisms show their higher efficiency of keratinase production at various pH ranging from 5 to 13[51]. Different enzymes produced by fungi, bacteria and other extremophiles combine with keratinase which are capable of highly efficient keratindegradation[52]. Biochemical and molecular studies of proteases indicate that enzyme engineering can be adopted for the development of disulfide boding between the keratinase for higher stability.

The mode of action of keratinase for keratin degradation is started with keratinase attachment to keratin. Keratin degradation by Keratinases is not site specific and keratinase attack peptide bonds of keratin at numeroussites. Keratinase can also cleave the bond between aromatic and nonpolar amino acids.

The most keratinolytic group belongs to fungi deuteromycetese including the species of *Acremonium, Aspergillus, Chrysosporium, spergillus, Alternaria, Trichurus, Ctenomyces, Curvularia, Cladosporium, Fusarium, Geomyces, Geotrichum, Gleomastis, Monodictys, Myrothecium, Paecilomyces,* *Pacecilomyces, Penicillium, Stachybotrys, Urocladium, Scopulariopsis, Sepedonium, Trichurus, Doratomyces.* Keratinase produced by these fungi has demonstrated effective cleavage of keratin substrate. More than 200 species have been used for the isolation of fungal keratinase. Fungal keratinase is very promising for the degradation of keratin and utilize it efficiently for degradation[53].

Dermatophytic species also have been reported to produce keratinase enzyme including *Microsporum canis, M. cookie, M. persicolor, Trichophyton krajdenii, T. mentagrophytes, T. raubitschekii, T. rubrum, T. Simii*and*Epidermophyton floccosum.*These reviews indicate that keratinolytic species are good sources of keratinase enzyme. [54]

Conclusions:

Keratinolyticmicroorganisms degrades the various keratinous waste in an efficient way. Degradation of keratinous waste materials by microorganisms reduces environmental problems in an eco friendly way. Biological degradation of keratinous waste is a simple and cheap method and dependent on some specific enzyme like keratinase. Keratinase enzyme along with some proteins, peptides and amino acids degrade keratin substrates efficiently. Keratinase from microorganisms can be used for recycling of poultry waste, animal feed and in leather industry.

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