Review Article

Production and Applications of Keratinases in Industry

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Abstract: Keratin is an insoluble protein with fibrous structure. It is mainly found in hair,feathers,nail,wool and horn of various animals. These animal accessories can be utilized as animals feed, amino-acids and fertilizers. This insoluble protein is very difficult to degrade and has extreme stability because it has disulfide bonds, hydrogen and hydrophobic interaction present in it. But keratin can be easily digested by keratinase enzyme. Keratinase is an extracellular enzyme. It also degrades keratin in prokaryotic organisms. Microorganisms that produce keratinase are species of Bacillus, Actinobacteria and fungi. It can also be obtained from Streptomyces, Aspergillus, Fervidobacterium, Xanthomonas, Chryseobacteriumand Vibrio. Keratinases produced by microbesarevery diverse due to their chemical as well as physical properties. It is a vigorous enzyme that can survive broad pH and temperature. Their optimum pH range is neutral to alkaline and their optimum temperature reported is 40°C to 60°C. And the rmophiles have also been reported for stable microbial activity. Microbial keratinases are cheaply available as compared to conventional producers for keratinases production. They have gained great significance in biotechnology by acting on strong, inflexible cross linked polypeptide structure of keratin instead of ordinary proteolytic enzyme papain, trypsin or pepsin. They are mostly extracted by degrading keratin as substrate. Keratin substrates include feather, hair, wool,horn and nails. This degradation process is helpful in converting keratinous wastes in to fertilizer and poultry feed. Other renowned biotechnological implementations include removing hair from leather by using keratinase, development of bio-polymer from keratinous fiber, delivering drug, hydrolysis of prion proteins and detergent industry. They are also used in converting biomass in to biofuel that significantly boosts power preservation and recycling. The main problem for producing enzymes on large scale is their costly production procedures. This hurdle is overcome by utilizing keratin waste eg feathers of chicken as fermentation substrate on industrial scale. The benefit of utilizing this left over is extremely cost effective fermentation process of keratinase and the effluents are environmental friendly. Keratinase enzyme has become popular and provided newly effective and efficient way for managing waste products by using them on industrial scales as substrates which gives rise to environmental friendly industries for nonstop development.

Keywords: keratin, keratinases, feathers feed, nitrogen fertilizer, biopolymerskeratin, keratinases, feathers feed, nitrogen fertilizer, biopolymers

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1. Introduction

Keratin is the major bio-polymer that is present in world in huge quantity [1].It is the fundamental component of structures of outermost layers of human skin, hair,nail and animal horns, bones, claws, beak and wool. It has a fibrous structure that is arranged in the form of monomers. When these monomers join they form intermediate filaments. It has extremely high stability and its breakdown is very difficult. This is because of presence of disulfide bonds, hydrogen and hydrophobic bonds in keratin [2].Keratin has two forms α keratin and β keratin. α keratin has alpha helix like structure and are present in hair, horn, wool,claws or nails and hooves of mammal. And β keratin has beta sheet like structure that are present in beak, claw and feather of birds[3].Keratin is insoluble in nature due to abundance of sulfur in disulfide bonds. Various amino acids are also present such as lysine, cysteine,serine and proline. It is very hard material that is composed of sclero-

protein and also non-reactive against many chemical therefore not digested by papain, trypsin and pepsin [4].In spite ofresistancekeratinous waste can be completely broken down by numerous bacteria,fungi and actinomycetes because of keratinolytic protease known as keratinase [5].

Keratinase can be serine or metalloprotease which breakdown fibrous and insoluble keratin containing material. They have broad temperature and pH ranges but their optimum functions are performed at neutral to alkaline i.e. 40 to 70°C temperatureranges. Thus they are vigorous enzymes having vast substrate specificity like keratin, hemoglobin, fibrins, gelatin and casein. But because no consistency in keratin substrate structures their abilities cannot be determined [6].Keratinophilic fungus produces proteolytic enzyme which has the capacity to decompose keratin containing leftovers [8].Keratinophilicfungi are basically Hyphomycetesthat can be further divided into dermophytytic and non dermatophytic fungi. Dermophytic fungi includes species of Microsporum and non dermatophytic fungi include species of Chrysosporium[9][10].Similarly like fungi, isolates of bacteria are also seen degrading keratin and also produced keratinases. The strains of bacteria include mainly of genus Bacillus e.g. *B.subtilis, B.licheniformis[*11].

Aditionally gram positive bacteria include Microbacteriumand gram negative bacteria include *Vibrio,Xanthomonas,Chryseobacterium* and *Fervidobacterium*are also reported degrading keratinous material [12].Keratinase is abundantly fermented in simple media having keratin containing substrate. Many microorganisms have capability to use keratin as only source which contain carbon and nitrogen [13].Main keratin accumulating origins are the reason or environmental issues initiating from many industrial sectors which utilize keratinous substances as raw materials. Poultry farm is dumps feathers waste e.g. barb or rachis. Almost 90 % of feather is made up of keratin and approximately millions of kilograms of bird feather have been disposed in environment every year [7].This discarded feather also includes naturally dropping of feather or hair from bird at the time of production. That is why it is important for developing process for reducing keratinous material addition in environment. To remove keratinous substances from environment instant, cost effective and easily performed process should be designed. Keratinases produced from microorganismshas the abilityto meet the desire and need because keratinophilic fungus, bacteria or actinomycetes needlessly inhabit keratinous substances [35].

In addition to recently discovered implementations of keratinases one extremely dominant is bio-conversion of poultry feathers into bio-energy. Poultry industrial sector produces huge amount of feathers wastes [14].But large amount of cost effective keratinases are pursued on industrial scale. Most scientists published micro-organisms that have ability to produce huge quantity of keratinases. Many experiments are performed for overproducing keratinases and reach the increased demand commercially [36].

2. Production of Keratinases

For keratinase production the accessibility and capability of many strains make selection of efficiently producing keratinase a fundamental first step. Then screening of microbial enzyme is very important for selecting as well as enzyme of each step specified should be cost effective, environmental friendly and effective.

2.1. In SubmergedFermentation

Keratinase produced by microorganisms are primarily extracellular enzymes when microbes are grown on keratin containing substrates [15].Mostly keratin acts as inducer. But non-keratin substrate e.g. soymeal can also induces enzymes [12].Then optimum parameters for producing keratinases by *Bacillus subtilis* strains requires 7.5pH initially, inoculation of 2 % (v/v), inoculums s age should be 16 hours and production temperature should be 23°C. It is recorded that maximal keratinolytic activity by KD-N2 strain was

accomplished after 30 hours. The essential amino acid seen producing were Threonine, Valine, Methionine and Ammonia while feather are utilized as substrate [30].Majorly it has been reported that for keratinases Sub-merged fermentation is preferred [6].Comparison of production parameters keratinases is complicated because of various keratinase producing microorganisms and their different method of production. Moreovercarbohydrate like glucose that is simple sugar has been recorded for suppressing production of keratinases. The reason is catabolite repression on the other hand while complex sugar e.g. starch has been reported to increase production of keratinase enzyme [16].

2.2. In Solid-stateFermentation

Solid substrate e.g. feather, hairs, horns or sugarcanes bagasse are utilized as inducer for producing keratinase from microorganisms by the process of solid-state fermentation [17].Likewise, reports show that adding 0.1 % of soy bean flour in feathers media increases keratinases quantity produced by using Bacillus speciePPKS-2 [18]Endo-phytickeratinolytic strains of Pencilliumspecies can outstandingly produce keratinases by utilizing solid substrate from various agriculture or poultry industry wastes[38].

3. Parameters of Keratinase Production

Biochemical and physic-chemical factors on which keratinases fermentation from microorganism depend has widely reported. Main parameters that deflect production are pH, temperature, molecular weight and substrate specificity.

3.1. pH

pH is one of the crucial factors that decide the development and morphology of microorganisms as they are delicate to the convergence of hydrogen particles present in the medium. Prior examinations have uncovered that organisms required somewhat acidic pH and microbes required unbiased pH for ideal development. pH is known to influence the union and discharge of keratinases like its stability [5].Most of the bacteria, fungi and actinomyceteshas optimum neutral to alkali pH ranging from 7 to 9 for development and enzyme production [31].Between many bacillus species mostly *B. licheniformis B. subtilis* and *B.pumilusshows* optimal activities at this pH [32].Therefore, keratinases produced by *B.subtilis* MTCC- 9102 has been found actively working in slight acidic pH [37].

3.2. Temperature

Optimum temperature range for keratinase varies with the change in sources or origins of different isolates. Basically optimal temperatures for keratinase production from various Bacillus sp. e.g. B.*licheniformis, B. subtilis, B. pumilus, B. halodurans,B.thuringiensis*and *B.cereusrange* among 40 to70 ^{o}C [33].Thermophiles microorganisms like Fervidobacteriumpennavoranshave optimum temperature 80^{o}C and 85 °C. Keratinase manifests diverse thermo-stability that ranges between 10 minutes - 36 hours at different temperature. Keratinase produced by bacillus has been reported stable at temperature range between 10^{o}C - 70^{o}C [37].

3.3. MolecularWeight

For keratinase production molecular mass has been recorded among 18 and 200 kDa. When bacillus species are used the size of keratinases varies between 20 to 69kDa.Smallest molecular weight reported is 18kDa for *Streptomyces albidoflavus* on the otherhand largest molecular mass is 240kDa reported for *K.rosea*. Mostly reports are of monomeric keratinases [37].

3.4. SubstrateSpecificity

Keratinase has shown vast substrates specificities. They have been recorded actively working in case of soluble as well as in-soluble protein substrate. As they have distinctive capacity of cleaving complexes, in-soluble substrate e.g. feathers keratin, collagen, elastin,fibrins and nails that show extreme resistance against decomposition by using traditional protease enzyme. Keratinase can completely hydrolyzes soluble proteins substrate e.g. casein,azocasein, bovine-serum albumin,gelatin [31].

4. Applications of Keratinases

Keratinase enzyme and keratinolytic micro-organisms can be used for diverse applications in various industries such as detergents, leather dehairing, food and feed production, medical and pharmaceutical industry and waste management also (Table 1). As compared to conventional microorganisms protease, keratinase can be obtained cost effectively by microorganisms. It has potential for using generous keratin containing wastes as carbon or nitrogen sources.Costly production is the major obstacle for economically producing enzymes. Therefore utilizing huge quantity of keratin containing wastes can extend method moreeconomically and expands implementations in various industries.

4.1. Bio-processing Poultry Waste forFeed

Feathers are mainly composed of keratin. It constitutes 90% of a feather weight that is 10% of complete chicken's waste [23].Recently feather is transformed in to feathers feed by the process of steam-pressure cooking that needs high power. Feathers feed is utilized as animals feed on restricted terms as ingredients because it has histidine,methionine and tryptophan deficiency [34].

Another possibility doe making feathers feed is hydrolysis by the help of enzyme of feathers by keratinase. Growth rate was compared among chicken fed with soya-bean feed and others fed with feathers feed that was fermented by Streptomyces species and Bacillus species and additionally adding methionine supplements. Crude keratinases from B. *licheniformisre*markably enhances overall amino-acid degradability and commercial availability as Versazyme[34].

4.2. Feather meal asFertilizer

Concentrated protein rich feathers feed produced poultry feeding can be utilized for biological farming as a semi slow releasing Nitrogen fertilizer. Biological or organic cultivation depends on using of nitrogen rich organic adjustments which provides two main advantages that includes improvement of growth of plants as well as increasing microorganisms in soil and their biological activity. Conventionally, organic cultivation was done by using Guano as fertilizers [27].Feathers feed is comprised of 15% Nitrogen that is very cost effective, easily accessible and acts as a worthy alternative to Guano. An advantage of feathers feed is not only nitrogen supply but it also enhances microorganisms activity, structure soil as well as increase H2O holding ability. Enzymatically hydrolyzing feathers feed has advantage over steam feed as fertilizer because it has elevated nutrition values, its effortless manufacturing and economical accessibility [12].Combining poultry feathers and plants leftovers altogether leads to upgrading of soil parameters and limits growth of phytopathogenic fungus Fusarium[36].

4.3. Detergent Industry

Protease enzyme is extensively used in detergent industries because it is a secure alternate that replaced hazardous chemical substances e.g. caustic soda. Recently most renowned brands of detergent industry add protease enzyme as major intermedial substance. Keratinolytic protease is more preferred as compared to ordinary protease due to their ability to react on stain surface of T-shirt collar or sleeves very efficiently [20].Keratinase has capacity for binding and hydrolyzing solid-substrates. It is key feature of the detergent enzyme because protein substrate bonded to solid surface requires their action. This makes these enzymes pleasing additive for cleaning hard surfaces.Keratinase can also be used for removing keratin containing soil which is frequently found in laundry like collar of shirt as many protease enzymes failed to remove them[12].Additionally keratinase can be utilized for cleaning drains [36].

4.4. X ray filmRe-ultilization

X-ray film is composed of Polyterephthalate (PET). It isextensively used for researches and medical assistance. Both of the sides of X ray film is coated with photographic emulsion and sensitive to light. This photographic emulsion of X- ray film is made of metallic-silver embedded in layer of gelatin. The utilized X ray films after Radiography are of great concern because they cause solid waste problem. The reason is presence of metallic-silver and plastic poly terephthalate in its construction. Huge amount of X ray film waste is disposed off in the backyard of every hospital, dispensary, pathology laboratories as well as small part can also be seen in ordinary municipal wastes [22].Conventional methods of recovering silver from utilized X ray film include ignition or acid treatment. These traditional processes of ignition recovers silver by combustingbutthis process don't retrieve polyterephthalate. And the drawbacks of traditional process are high cost and formation of harmful byproducts like smoke. In the same way treating it by acid includes separation of layer of gelatin using robust acid solution. This generates drawback of acid effluent production after recovering silver [22].On the other hand, keratinolytic enzyme provides environmental friendly process for recovering silverfrom X-ray film. This is due to their ability to utilize gelatin as substrate and results in releasing metallic-silver in solution. This process also recovers Para terephthalate base unaltered [22].

4.5. LeatherIndustry

Amount of pollution produced during leather production is usually in pretanning step. It constitutes approximately 70% of total hazardous effluents of the industry. Sodium- sulfide, lime and solid waste products that are produced as end product of pretanning are chiefly involved I increasing Biochemical Oxygen Demand (BOD) and increased Chemical Oxygen Demand (COD) [24].Keratinolytic protease lacks collagen lytic but has gentle elastolyticactivity. They are mostly studied for increasing the efficiency of de-hairing process. They have been used for selectively breaking keratin tissues present in follicles and allow intact hair to be pulled out so the tensile strength of the leather is not affected[25].

4.6. BiofuelProduction

Bio-energy can also be produced by using poultry wastes. Keratin degrading microorganisms and keratinase enzymes has also used for generating Natural gas, Methane gas, Pellet fuels and producing Biohydrogen [36].Keratinous end products can be converted in to fuel and can potentially lead to increasing interests for preservation of energy and recycle process. Bio-diesel can also be produced by using poultry wastes [26].

4.7. Degradation of Pathogenic PrionProtein

Infectious agents like prions are made up of proteins that are present in misfolded forms. Prion word is combined of protein and infection. PrP is the main protein also known as proteosome resistant protein. It is usually expressed in nervous system [34]. They cause incurable neuro-degenerative disease known as transmissible spongiform encephalopathies (TSE). This disease includes Dreaded cow disease, Scrapie, Kuru and Creutzfeldjakob diseases [12].

It has been researched that broad-spectrumkeratinases known as versazyme can efficiently degrade complete prion from brain tissues in Bovine Spongiform Encephalopathy (BSE) and also from Scrapie infected animal by giving heat treatment and heat treatment and adding detergents. Due to this research we can conclude that if enzymes can break down prion, they can also be proved helpful in de-contaminating medical and laboratory apparatus and other replaceable items such as contact lens or dentist'sequipment[28].

4.8. NematodeSuppression

Nematode is a parasite that affects crops production. It causes failure of crops worldwide. Various harmful substances have been manufactured by agricultural chemists e.g. nematicide. They are used for minimizing loss of crops due to plant- parasite nematode. Not long ago some research has been successfully conducted for using keratinases as pesticides for controlling root-knot nematode also known as Meloidogynespecies[29].Keratinase was obtained from Bacillus species and purified for evaluating prevention off Meloidogyne incognita. It has been observed that nematodes cuticle wasdegraded after treatment given for 24 hours. This nematode cuticle was composed of keratin and collagen protein. And this research proved that keratin can be safely used as an alternate for hazardous chemical pesticide [29].

5. Conclusion

Now-a-days, environmental pollution is of great concern and if any of waste product is successfully degraded on industrial scale as keratin, it becomes the topic of interest for researchers. Keratinase has boosted the annual income of various industries e.g. poultry, textile, leather, bioenergy and pharmaceutical industry etc. But there is still need of environmental friendly technique for overcoming some of stubborn keratinous waste products to preserve nature. Therefore researchers have been reporting various keratin containing waste products that can be used in their raw form as substrate and keratin degrading microbes to replace conventional methods.For overcoming these problems objective of research should be to improve parameters of already existing keratinase or search novel keratinase producing microorganism that has broad substrate specificity

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Table 1. Characteristics and applications of different keratinase producing microorganisms

Microorganisms	Catalytic type	Optimal pH	Optimal temp. °C	Molecular weight kDa	Substrate Specificity	Applications	Ref.
Bacillus Sp. SH-517	Stillnot known	7.5	40	51	Casein	Leather	[20]
Brevibacillus Sp. AS-S1OI-I	Still not known	12.5	45	83.2	-	Leather, Detergent Industry	[39]
Bacillus spp	Serine	10	40-50	Approx. 200	Feathers, gelatin	Bio-degradation of feathers	[40]
Bacillus subtilus NRC-3	Metallo	7.5 or 8	40 or 50		Feather keratinazure	Treating feather waste	[41]
Paenibacillus-woosongenesis TKB2	Still not known	9	50	90.24	Feather	Detergent industry	[42]
Streptomyces albus	Serine	7	40	29 to 35	Hair	Degradation	[43]
Kocuriarosea	Serine	10	40	240	Keratin, collagen,Gelatin and casein	Recycling poultry feathers	[44]
B.cereus1268	Serine	10	40-50	200	Feather	Degradation of feathers	[40]
P.aeruginosaSK1	Serine	9	60	45	Feather, fibrin, inoculums and meat protein	Degradation of prions	[45]
Stenotrophomonas sp.	Serine and disulfide reductase	8	30	40	Casein, human hair, bovine hoof, collagen	Degradation	[46]