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Review: Keratin and Keratinase

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I. Keratin source in leather Industry

Feather and leather processing industry are involved in generation of huge amount of waste, chicken Feather are found to be very rich source of amino acids, peptides, because these contain 90 % protein, (Onifade et al. 1998). Annually there are 5 million tons of feathers are processed in poultry industry every year, Tannery industries uses greater amount of chemicals like soda lime, salts chromate sulphide during water processing. Their occurrence are also seen in the form of emulsified fatty matter, waste lime liquor. A long time exposure of biological waste from leather industry will cause environmental pollution (Pepper and Wyatt et al,1989).

Keratin are good source for insoluble, fibrous material which are highly rigid molecules and found as recalcitrant. These poly molecule exhibits non degradable characteristics in the nature and stay for longer period in environment.

Chemical used in leather processing industry are of highly corrosive in nature and cause high amount of health hazardous. Some tanneries are closed down due to their high amount of pollution in environment (Davighi et al, 1988).

The traditional leather processing industry is becoming a great concern owing to the large amount of waste water and 700 kg solid trash.

Proteases obtained from alkaline source highly effective in removing hair from the skin of this hair contain keratin protein with a rich amount of sulpher bond, and which exhibits hydrophobic nature. This leads to decrease in the unhairing efficiency, it was found that kerationlytic protease had great potential in enzymatic processing .

Sulfide treatment is becoming more failure approach due to heavy pollution, caused during unhairing. keratinase shows environmentally friendly stand. (Zhen Fang et al, 2017)

Class of microbiota like bacteria, actinomycetes, fungi, dermatophytes produces keratinase from keratin source.

Keratin fibers contain protein amino acid predominantly like cystine lysine proline and serine. Considering the secondary conformation of amino acid keratin structure are classified as alpha helix majorly found in hair, wool. Beta keratin sheets are found in feathers,

Keratin is the integral structure of animal parts like horns and hooves, cornfield skin. These protein exhibits high mechanical strength, their proteolytic activity of microorganisms have the ability to degrade native keratin in nature. (Kim et al. 2001; Grazziotin et al. 2006; Khardenavis et al. 2009).

Trivalent Chromium (CrIII) salts along with sulfate are currently most important tanning agents, every raw hide processed will generate large amount of Chromium tanned leather Shavings (CTLS) waste, In China alone CTLS reaches 7,00,000 metric tons, enzyme hydrolysis of Cr-free solid waste been studied. Chromium tolerant bacteria strain isolated from tannery soil along with keratinase, collagenase, proteinase enzyme in post tanning help cost reduction and protect environment. (Shan Cao et al, 2017)

The most prominent Source of keratin is feather meal a byproduct obtained from the poultry processing unit, which has millions of turn over annually through out the year. At present poultry waste are generated by steam, chemical treatment. Chicken feather can generate high amount tryptophan after degradation, these are all good source of biofertilizer, serves as cheap source for nitrogen (15%). The nutrients obtained from feather degradation, facilitate the growth of plants by promoting the activity of protein hydrolysates. (Siddharthan Nagarajan, et. al.)

For degradation of hooves and horns Hydrothermal pretreatment was proven to be more effective with the bacterial and fungal keratinase.

Keratin is a highly insoluble fibrous protein, on hydrolysis release variant amino acid cystine, lysine, proline Serine, protein secondary structure will determine them has soft and hard keratin. Fungi play a important role in maintain ecological role by release carbon nitrogen and sulfur.

Due to Beta- keratin structure it is hard to degrade feather meal, human hair, feather, nails, sheep wool, So *fervidobacterium* species was cloned and recombinant strain islandicum curde extract was subjected for LC-MS/MS to detects keratin. Similarly keratin degrading and keratinase expressing gene are encoded in the following microbes *Bacillus licheniformis ,Streptomyces pactum , Fumigatus fresenius, Trichophyton mentagrophytes.* (Hyeon-Su Jin et al, 2017)

During the production of feather meal there is possibility of more protein and enzyme loss, but Biodegradation of poultry waste become a big challenge for poultry industry. This problem can be managed by traditional method such as land filling, incineration chemical treatment. (Siddharthan Nagarajan, et. al, 2017)

II. Keratinase

keratinase application is also seen in upcoming area like bioprocessing of used x-ray film, glue and foils and agro industrial waste degradation. (Saber et al. 2010)

Different engineering techniques like exogenous signal peptide, Computational design and empirical mutation, C-terminal domain partial truncation, functional domine exchange, strategy are applied to develop microbial keratinase such as *B.coli, B.Subtilis, Streptomyces fradiae*. (Fang et al. (2013a), Liu et al. (2014))

Various microorganisms such as *Bacillus, pseudomonas* and *trichoderma* are very usefull organism in facilitating plant growth and solubilization of nitrogen fixation and biocontrol of pest.

Several set of microorganism exhibit keratinase activity and are able to degrade feather meal, theses microbes with enzyme release free amino acid. Most of these enzyme are thermophlic in nature they differential expresses keratin degrading c-DNA for synthesis of keratinase enzyme. Organism such as *F. Islandicum AW-1* and the *Gallus gallus*, *Escherichia coli* Curd extract of these organism are used to test the activity.

keratinases group of serine or metalloproteases. These enzyme are predominantly produced by microorganism growing in a basal medium containing keratinous substrate. The cysteine amino acid in the keratin considered as protease with keratinolytic function act synergistically with keratinases enzyme considered as supramolecular organization of keratin waste can be efficiently degraded by various microorganism.

Kerotinolytic enzyme are treated as green biocatalyst for leather industry.

Keratinase is replacement for all kind of proteases. degradation is facilitated by bacterial species like *Bacillus licheniformis*, *bacillus subtilis*, chryseobacterium, fungal species such as *Aspergillus, penicillium, Microsporum, Trichoderma, Chrysosporium* genera perform keratinolytic degredation and their effect was analysis using Scanning Electron Microscope (SEM) Foureir Transform Infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA). (Zhen Fang et al, 2017)

Keratinase has large application in very broad area like detergent, enhancement of drug delivery system are emerging application in medical, cosmetics, pesticides.

The compost prepared using keratinolytic Bacteria will promote faster plant growth, and resend trended shows use of synthetics fertilizer for scientific methods of crop improvement was highly preferred, Using Plant Growth Promoting (PGP) bacteria approach is most accepted technique by agronomists and environmentalists. PGP System is Highly economics system to increase yield and cater the growing population, usage of Chicken Feather Compost in addition with *Bacillus, Pseudomonas* and *Trichoderma* have been considered as potential plant growth-promoting factors.

Chicken Feather Meal is Good source of nitrogen, Scientific source have shown nitrogen release during degradation, which is a enrich source of tryptophan and further synthesis of Indole Acetic Acid IAA.

Feather Compost were subjected for microbial degradation and Physical chemical parameters were tested like pH, water holding capacity, permeability, soil moisture, salinity, acidity,temperature, conductivity minerals like N, K, Mn, Zn, Mg, Na, Cu etc .

Growth of plants were tested in leaf length, Shoot, root induction were checked.

(Siddharthan Nagarajan, et. al, 2017)

Application of keratin

Keratin obtained from claws, turtle scutes, horn, baleen hair, beak , feather ,scales to armor body are greater epidermal appendage. Some are categorized has dead tissue, and some have amorphous structure due to embedded alpha and beta keratin structured,

If we check the keratinized cell are microscale lamellar, tubular, porous core, compact sheats sandwich young's modulus (Gpa) and tensile strength were checked .

Comparison of alpha keratin and beta keratin were beta was more in plateded sheath, amino compostion of feather and wool are compared, phase transistion ,viscosity shear stress strain, structural reframent tensile force displacement, deformation studies, energy absorption, linear elastic studies, hydration studies on water uptake, relative humidity.

Scaning Electron Microscope of Stratum corneum of human skin, human hair cuticle, micrographs of porcupine and quill ,bovin horn sheath ,dorsal intermediate ventrials surface fingernail, baleen plate, sie, minke, hagfish Slime threads (bundles of keratin Ifs),whelks string egg capsule, seagull feather, turtle shell pangolin scale. (Bin Wang et al 2016)

Today keratin and Keratin hydrolysate have found application in following area Biofuel (Biohydrogen), Bio fertilizer, Bioremediation (removal of lead), chrome tanning effluent, Cosmetics (Shampoo, Elastin).

Commercially available keratinase product :-

1.Versazyme (keratinase from B. licheniformis) BRI Recycling of keratin waste.

2.Valkerase (keratinase from B. licheniformis) BRI.

3.FixaFungus™, FixaFungus Treatment of nail disorders.

4.Kernail-Soft PB (keratinase from B. licheniformis) Proteos Biotech.

5. Pure100 Keratinase (keratinase from B. licheniformis) Proteos Biotech, 6. Keratoclean® Hydra PB.(Rani Gupta et al, 2013)

III. Production of Recombinant keratinase

Genetically Engineered Bacillus licheniformis using insect cells/ baculovirus expression system with the insertion of Keratinase A gene ligated to pUC57 transforming into pFastBac1-OKer-His-Flag into *E. coli* DH10Bac™ cells construct. This cloned microbes was used to test pH, temperature keratinase enzyme has the final activity of 635U/mg. The insect cell baculovirus expression vector system (BEVS) is responsible for high level of intracellular expression with post-translational modification for exogenous gene. (Miaorong Huang et al 2017)

New strain such has *Aphanoascus fulvescens* and *Chrysosporium articulatum* were isolated from soil, identification of strain was done based on phenotypic trait, the studies involves Usage of Response Surface methodology for increases and optimize highest keratinolytical activity, experiment involves use of BOX-Behnken design for the loss of substrate feather meal. This loss is incurred due to enzyme purification step, were ammonium sulfate are used, due to its alkalizing nature the enzyme keratinase will get inhibited. (Justyna Bohacz, 2017)

Utility of feather meal has soil fertilizer by keratinase enzyme enhance biogas, composting lead to anaerobic cultivation of bio methanation a environmentally efficient approach and fermentation. Hydrothermal pretreatment is proven to be advantage for hooves and horn prior to enzyme treatment, peptidic solution contain pool amino acids obtained from pig bristle, wool by B. Pumilus. B. Subtilis S1-4 was a source of specific antioxidative peptide.

B. Cereus stain in decomposting feather and generation of serine proteinase. Keratinase activity on keratin hydrolysis, Box Behnken design FTIR measurement are performed. (Wojciech Laba et al, 2017)

Gallus gallus chromosomes contain chromosome for beta keratin 2 and 27 this recombinant protein substrate were used for enzyme activity, *Fervidobacterium islandicum* AW-1 recognize wide range of substrate like casein, protinase k, trypsin, papain, keratin hydrolyzed crude extract was used for LC-MS/MS. *Gallus gallus* genome has revealed details of the genomic evolution, development, expression of keratin protein. (Hyeon-Su Jin et al, 2017)

Fearther meal preparation was carried out by colleting chicken feather from poultry house, then washing with soap and water to remove blood stain and dirt. Dried under sunlight for 24 hours. The feather were chopped for small pieces by scissor of length 1-3 cm long. Fefatting of feather was performed by using chloroform:methanol (1:1) for 2 days followed by chloroform:acetone:methanol (4:1:3) for 2 days solvent was replaced every day, dried in oven 40^0c for 24hr and blending gives feather meal. (RABAB OMRAN, 2017)

Melanin is distributed in animals, this embedded in keratin matrix that make feather more resistance to the insoluble degradation for *Chryseobacterium sp*. It help in growth survival and competitiveness, it is dark

color and negatively charge higher heterogeneous polymer *Bacillus licheniformi* was unable to degrade melanized feather, *Xanthomonas maltophilia* was able to degrade keratin to carbon , nitrogen, sulpfur.
In this experiment the keratinase activity was showing 21.9 Uml-1 at 12 hr and highest at 24 hr 89.12 ml-1 and decreased again 120 hr 16.20 U ml-1during the process pH shifted from 7.5 to 8.8. alkalinity is due to deamination of media alkalinity and amino group. *Streptomyces pactum* performed sulphlytical activity .(Ranjit G. Gurav et al, 2016)

The biological Pre treatment of feather meal was improved to yield more methane biogas, hydrolysate of curde protein yielded 445 ml of CH4/g. untreated feather subjected for total solid test, nitrogen, moisture C/N Fat content Bulk density test. Microbes like *B. megaterium, Bacillus licheniformis* were used in investigation feather was left for 6-8 days for degradation. (Regina J. Patinvoh et al, 2016)

Bacterial Strain was isolated and purified using Liquid chromatography- electronspary ionization tandem mass spectrometry (nano HPLC-ESI-MS), DNA sequence of ORF Open Reading Frame Ker1, Ker2 gene contain 1153bp with sequence similarity of 67% in *Bacillus pumilus* strain was searched in Genebank, Protein Peptide of keratinase enzyme 38.8, 38.3 KDa isolated using SDS-PAGE. (Soltana Fellahi et al, 2016)

Microbial degradation was better seen in new isolates obtained from the novel lime stone source, more keratinase production was delivered when citrate and soybean source given to the enzyme producing media. *Bacillus sp.* MBRL 575 producing keratinase enzyme was identified and phenotypic characterization by 16S rRNA sequence. This organism has exhibited antagonistic activity of fungal strain (Pintubala Kshetri et al, 2016) The pathogenic Non Sporulating Molds NSM fungi *Ascomycota* and *Basidiomycota* (*Trichophyton, mentagrophytes, Trichophyton rubrum, Microsporum* canis and *Fusarium* spp.) has good keratinolytical activity (7.13 ± 0.552 keratinase units) than the non-pathogenic (2.37 ± 0.262 keratinase units) activity. (Nantha Kumar Jeyaprakasam et al, 2016): (Panchanathan Manivasagan et al, 2014)

Bioinformatics of Keratinase

The fungi isolates of species *Ascomycota, Basidiomycota, zygomycota* their ITS1, ITS2 and 5.8S r region were identified, theye are isolated on SDA (Sabouraud dextrose agar) media, characterization of fungi was done PCR, sequencing, NCBI Blast DNA databank , pair wise alignment , EMBOSS matcher, similarity for 18sRNA was searched keratinase activity was graded, phylogenetics tree was constructed using neighbor joining method for all category of strain isolate. (Shiv M. Singh et al, 2016)

Stenotrophomonas maltophilia enzyme is having high stability for pH, catalytic efficiency K_{cat} /K_m 143.6 $S^{-1}mM^{-1}$, due to presence of alkaline stability collagenase activity is decreased and partially truncated keratinase is increases. thermophilic nature 60^0C and 1.7 fold increased activity salt and detergent resistance is seen in KerSMD.
(Zhen Fang et al, 2016)

Acinetobacter sp. Produced more keratinase in the presence of Li^+, Na^+ and Ca^{+2} while enzyme get inhibited by EDTA since it is metallo keratinase obtained from poultry contaminated soil. surface modification of wool hair was a evident for supernatant contain keratinase.
(Rong-Xian Zhang et al, 2016)

Mesophilic keratinolytic was increased by deleting and replacement C-terminal domain generation of mutant peptide, which with stand *Tm* value of 64.60 ± 0.65°C and a half-life of 244.6 ± 2 min at 60°C,
Optimization of keratinase gene from recombinant *B. subtilis* and *P. pastoris* has
3,010 and 1,050 U/mL highest activity ever reported by bacterial recombinant strain and it was cultures in 3 lt fermenter, pH was 10 for Ker E, Ker B, Ker P.
(Baihong Liu et al, 2014)

Recombinant rK27 was purified using affinity chromatography which in turned showed 80 % recovery. Bone meal was used as substrate and its hydrolysis was flocculated after keratinase treatment and ProteinaseK. (Rinky Rajput et al, 2014)

Paenibacillus woosongensis is a feather-degrading microbes cultured on Solid State Fermentation produces keratinase enzyme which act on goat hide surface during dehairing, RSM Response Surface Methodology was used for optimization.
(Tanmay Paul et al,2014)

Bacillus subtilis S14 showed Maximum keratinase production was obtained with aeration rate of 1 vvm, agitation of 400 rpm and an oxygen mass transfer rate of 24.48 h−1. (Lucas Andre Dedavid e Silva et al, 2014)

Actinoalloteichus sp. MA-32 growth media was designed using Plackett– Burman (PB) design and it was applied to find the key ingredients and conditions for the best yield of keratinase production and central composite design (CCD) used to optimize the concentration of all the five significant variables such as whole chicken feather, soy flour, $MgSO_4$ $7H_2O$, KH_2PO_4 and NaCl.
(Panchanathan Manivasagan et al, 2014)

Aspergillus terreus showed promising keratinolytic activity on solid state fermentation with 5gm wheat flour bran as substrate, 75 % saline 677 U/ml for 72 hr pH 6.5 temperature 55 ^oC
(Ana Claudia Rodrigues de Siqueira et al, 2014)
Bacillus subtilis is efficient microbes in degrading poultry feather waste, the fermented media carbon source used was soya bean meal. Culture filtrate showed cysteine, cystine, methionine, and total free amino acids during the fermentation period.
(P. Jeevana Lakshmi et al, 2013)

Microsporum fulvum IBRL SD3 is one of new isolate for chicken feather degradation.
(I. Darah et al, 2013)

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