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Review

Biodegradation of Feather Waste: A mini review

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Abstract

Feathers make up about 5% of the body weight of poultry. It is a considerable waste product of the poultry industry being produced over 8 billion tons per year worldwide. Accumulation of waste feathers result in keratin wastage and environmental pollution of land and underground water resources. These proteins can be sustainably utilized with the help of several microorganisms. Biotechnological methods employing keratinolytic microorganisms play a key role in processing keratin waste. This study reviews the current knowledge on the keratinolytic microorganisms and presents the multiple applications of keratin degrading enzymes. Also the requirement of biotransformation of keratin rich feathers into products of agricultural and industrial value is discussed.

Keywords: Biodegradation, Feather keratin, Biotransformation

Introduction

Chicken feathers are the major waste product of the poultry industry being discarded in bulk as waste from poultry processing industries, poultry farms and shops, globally. Billions of kilograms of waste feathers are generated each year by poultry processing plants, creating a serious solid waste accumulation problem (Parkinson G., 1998; Schmidt W.F., 1998.). As a resource-saving treatment of the environment, feather waste should be recycled which ensures that it can return to the value-added cycle once again and thus could be utilized resourcefully. Feathers; making up about 5% of the body weight of poultry is being produced about 8.5 billion tons per year worldwide (Sarita Agrahari and Neeraj Wadhwa, 2010). Chicken feathers are structured as barbs (feather fiber) and rachis (quill) which forms the central core with hollow tube like structure. Both feather fiber and quill are made of β-keratin, an insoluble and highly durable structural protein found in nail, hair, hoof, and horn of animals (Karshan 1930; Schmidt 2002). One of the main characteristics of keratins is that they have high mechanical stability and resistance to proteolytic degradation, which depends on the disulfide, hydrogen and hydrophobic bonds, salt linkages and other cross-linking. (Kaluzewska *et al.*, 1991; Friedrich and Antranikian, 1996). Among these the disulfide bonds are strong covalent bonds which stabilize the three-dimensional protein structure and thus are difficult to breakdown (Alberts, B; 1994). The β-sheet structure is a challenge for many of the enzymes. This can be the reason why keratinous materials are tough and fibrous being mechanically firm, chemically non-reactive, water insoluble and protease-resistant. Such a molecular structure makes feathers poorly degradable even in anaerobic condition. Due to their insoluble nature; feathers are resistant to degradation by common microbial proteases (Onifade *et al.*, 1998). They are not recognized as substrates to common proteases (Letourneau et. al., 1998). It is difficult to initiate the degradation process as they are covered by a fine powder or oil secreted by the birds which make them water resistant. Since feathers are almost pure keratin protein, feather wastes represent a potential alternative to more expensive dietary ingredients for animal feedstuffs (Shih, 1992). However, feathers are currently utilized on a limited basis as a dietary protein supplement for animal feed because feather meal production is an expensive process, requiring significant amounts of energy. In addition this process destroys certain amino acids, yielding a product with poor digestibility and variable nutrient quality (Papadopoulos *et al.*, 1986; Wang and Parsons, 1997). The drawbacks of high temperature treatment gave a way to use microbial keratinases that serve as attractive alternative to hydrolyze feather. Thus there is always a requirement of isolation of enzymes from new sources to meet the industrial and environmental demand (Gupta and Ramnani 2006). The traditional disposal strategies of chicken feather are difficult and even expensive. They are often disposed in incineration plants, buried in landfills, or recycled into low quality animal feeds. However, these disposal methods are supposed to generate green house gases that pose danger to

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the environment. Thus the increased need for energy conservation and recycling along with the increase in poultry industry has strongly stimulated the search for potential microorganisms capable of degrading the keratinous waste. Developing countries including India should recycle the highly proteinaceous waste generated from poultry industry because of the increasing need for improvement of their socio-economic status.

Structural Integrity of Feather Keratins

Keratins are insoluble structural proteins present in feathers, wool, hooves, scales, hair, nails which are hard keratins and in stratum corneum which forms soft keratins. These specific proteins which belong to the scleroprotein groups are compounds that are highly resistant to physical, chemical and biological actions. They are very complex due to the multiplicity of protein molecules which are effectively cross-linked to form an integral structure. The chains of polypeptides are cross-linked by means of S-S bonds of cysteine. The chemical contents of feather, as percentage of dry weight are protein 85.4%; fats 1.22%; Ash 8.6%; fibers 0.68%; calcium 0.55%; and phosphorus 0.16% (Han and Parsons 1991, Riffel and Brandelli 2006). Keratin consists of a number of amino acids but largely made up of cystine, arginine, threonine, lysine, proline, and serine. (Ward *et al.* 1955; Harrar and Woods 1963). These amino acids tend to cross-link with one another by forming disulfide or hydrogen bonds resulting in fibers that are tough, strong, lightweight, and with good thermal and acoustic insulating properties (Schmidt 2002; Poole *et al.* 2009). Intercellular hydrogen bonds are present in the conformation of the β-type polypeptide chain. They fix antiparallel peptide chains which stabilizes the protein structure (Filipello Marchisio, 2000). Polypeptide chains in β-keratin can be parallel as well as perpendicular (cross-β) to the filament axis. Feather keratin, the third type of keratin, occurs in both configuration systems of the polypeptide chain: α-helix and β-sheet of which β-conformation proteins in 1/3 and α-keratin in 2/3. 90% of feather keratins are formed by homogeneous proteins with a molecular weight of 10.4 kDa (Lee and Baden, 1975; Fraser *et al.*, 1972). All these contribute to the structural rigidity and integrity of feather which makes it a hard to degrade protein.

Microbial Keratinolysis

The degradation of keratins is attributed to an inducible enzyme keratinase secreted by several microorganisms. With the help of keratin degrading enzymes feathers could be converted to rare amino acids like serine, cysteine and proline as well as useful peptides. Keratinases are produced by a wide variety of microorganisms that include bacteria, actinomycetes and fungi. Over the past decade there were several reports on the characterization of keratinases from several organisms including those of *Bacillus sp., Streptomyces sp., Chryseobacterium sp.* and *Stenotrophomonas sp.* (Prakash *et al.*, 2010; Coa *et al.*, 2009). Keratinases from microorganisms have attracted a great deal of attraction in the last decade particularly due to their various industrial applications. The particular ability of keratinolytic proteases may be due to specificity for compact substrates and a more exposed active site. The complex mechanism of keratinolysis involves the combined actions of sulfitolytic and proteolytic systems. The enzymatic cleavage of the peptide bonds of keratin is difficult because of the restricted enzyme-substrate interaction on the surface of keratin particles. The production of keratinases has been a domain of mesophilic fungi, actinomycetes, and some Bacillus species (Williams *et al.*, 1990; Lin *et al.*, 1992; Takami *et al.*, 1992; Bockle *et al.*, 1995). Keratinolytic protease enzymes are widely secreted in nature by different groups of microorganisms that can be isolated from areas polluted with keratin wastes (Gupta and Remnani 2006). A vast variety of Gram positive bacteria including Bacillus, Lysobacter, Nesternokia, Kocuria, and Microbacteriumas well as a few strains of Gram negative bacteria such as Xanthomonas, Stenotrophomonas, and Chryseobacterium are confined as keratin degraders (Sangali and A. Brandelli, 2000, C. H. De et. al., 2002, Lucas *et al.*, 2003, Yamamura *et al.*, 2002). Most of keratin degrading bacteria belong to the genus of Bacillus (Brutt and Ichida, 1999). Along with bacteria and fungi, some insects including cloth mouth leaves, carpet beetles are known to digest keratin (Bin Zhang *et al.*, 2009). Keratinases are inducible enzymes meaning which they are produced only in the presence of keratin containing substrate. It mainly attacks on the disulfide (-S-S-) bond of the keratin substrate (Bockle *et al.*, 1995).

Keratinases are proteolytic enzymes in nature with EC number 3.4.99. It was classified as proteinase of unknown mechanism as recommended by the Nomenclature Committee on the International Union of Biochemistry (1978). Recently, some of the researchers defined keratinase as serine protease due to its 97% sequence homology with alkaline protease and it is also inhibited by the same inhibitor that inhibits serine protease (Wang and Parsons, 1997, and Bressollier et a1., 1999). Keratinases are primarily extracellular in nature, but a few cell-bound and intracellular enzymes have also been reported (Kim et. al., 2002, El-Naghy et. al., 1998 and Onifade, et.al., 1998). They are mostly serine proteases, showing substantial sequence similarity with subtilisins; thus they are considered as keratinolytic members of subtilisin group of proteases (Gupta and Remnani 2006). Metalloprotease are also found with specific keratinolytic activity from *Microbacterium sps.* (Brandelli and Thys, 2006), Chryseobacterium (Wang et. al., 2008) *Bacillus sps.* (Lee et. al., 2002, Kainoor and Naik 2010). Keratinolytic proteases mostly belong to serine or metalloproteases showing sequence similarity with subtilisin group of proteases (Dozie *et al.*, 1994, Riffel *et al.*, 2003). Because of environmental considerations the use of keratinolytic enzymes in the degradation of keratins is becoming attractive

for biotechnological applications. The enzymatic process is advantageous over commercial methods, as large amounts of salts, which need to be separated from the end product, would not be produced. Also, biological treatment improves the nutritional value of feather waste and is environmentally friendly. (Safranek and Goose, 1982; Sohair and Assen, 1974; Giongo *et al.*, 2007; Linn *et al.*, 1995; Ramnani *et al.*, 2005).

Applications of Keratinases

In recent years, more demands to keratinolytic proteases are increasing due to their multitude in industrial applications. Microbial keratinases are considered as potential biocatalysts for several purposes, including applications in feed, fertilizer, leather and textile industries. Keratinase producing microorganisms have the important industrial application in fermentation technology. Submerged fermentation of poultry waste by microorganism producing keratinase helps in the conversion of non-soluble keratin (feather) into soluble protein or polypeptide (Suntornsuk and Suntornsuk, 2003). These protein byproduct may be used as animal and livestock feed, and as leather filling agents (Sastry *et al.*, 1986). Also the enzyme hydolysate which is supposed to contain useful peptides and aminoacids can be utilized as organic fertilizer. Keratinase has also emerging application in dehairing process in leather industry instead of sodium sulphides (Alexandre *et al.*, 2005) and also used as detergent to remove strains on clothing (Gessesse *et al.*, 2002).

Keratinase can degrade all the protein molecules, so it may also be used in detergents. This enzyme can be an alternative to sodium sulfide, the major pollutant from tanneries, or may completely replace it. Its unique non activity upon collagen enhances its industrial potential (Alexandre *et al.*, 2005). It is therefore easy to convert the feather keratin into soluble crude protein through microbial fermentation technology by using pilot scale bioreactor (Singh, 2007) and soild state aerobic and anaerobic reactors to yeild byproducts (Rajesh Banu *et al.*, 2006). Adetunji 2012 made studies on the effect of keratin based organic fertilizer from microbially hydrolyzed feathers on the growth of cowpea (*Vigna unguiculata*) and was found very effective with better yield and productivity. It is also has a wide range of application in pharmaceutical and biomedical sectors (Onifade *et al.*, 1998, Gupta and Remnani 2006 and Brandelli *et al.*, 2010). Keratinase from *Bacillus licheniformis* strain PWD- 1 has been detected as having prion protein degradative nature in the brain and animal tissue (Selvam and Vishnupriya 2012).

A combined treatment of cutinase, keratinase and protease was applied in the wool processing to modify the wool properties. This combined action of this enzyme on wool has improved the wetability and anti-felting property of wool fabrics. Here, keratinase could reduce the high degree of cross linking in keratinous proteins and make the action of protease on the wool surface easy. (Ping Wang *et al.*, 2011). Keratinase hydrolysed the outer epithelial sheath of hair roots provoking depilation which suggests the potential of the enzyme for application in eco-friendly leather processing (Weitzman and Summerbell, 1995). The enzymatic disruption of nail plate using keratinase has been studied by Robert Preston (2001) for enhanced drug penetration into the nail plate and was found that the hydrolytic action of keratinase on nail plate proteins could effectively increase the drug delivery without harming the nail. This finding gave a way for the pharmaceutical enhancement of nail treatment. The capability of extracellular enzymes to penetrate through the solid structural barriers in the host is being utilized for the treatment against fungal pathogens. Keratinases are used in dermatophytosis therapy in humans, pets, sheep and cattle (Lin *et al.*, 1992).

Keratinase from *Bacillus licheniformis* PWD-1 and E.coli has been adapted to the laboratory and cosmetic applications especially in acne treatment (Saha and Dhanasekaran, 2010). Too much of dead cells can choke pores and create a favorable environment for development of acne. The keratinolytic property of keratinase has now been applied in the treatment for acne. Several facial scrubs available in the market now-a-days include keratinases because of its effective action for the treatment for acne. Some of the anti-dandruff shampoos also include keratinase because the keratinases act upon the scalp to wash away flaky dead cells. It is also been used to eliminate warts, calluses and corns. The U.S food and drug administration (FDA) has approved keratinase in the treatment of psoriasis, a condition involving excessive turnover of skin cells and scaly build up (Veslava *et al.*, 2009, Vigneshwaran *et al.*, 2010). The protein rich, concentrated feather meal can also be used for organic farming as nitrogen fertilizer (Kainoor and Naik, 2010). The application of keratinase in the improvement of feather meal and feed additives has lead to the commercialization of feather meal as a high protein feed as "Versazyme" (Odetallah *et al.*, 2005). The production of amino acids or peptides from high molecular weight keratins finds its application in the cosmetics industry (Mazotto *et al.*, 2011; Cedrola *et al.*, 2012). Also, keratinases have been associated with to prion degradation and investigated as an active pesticide against root-knot nematodes (Mitsuiki *et al.*, 2006; Yue *et al.*, 2011. Microbial keratinases exhibit great diversity in their biochemical properties with respect to activity and stability in various pH and temperature ranges (Brandelli *et al.*, 2010). Bacterial and fungal keratinases are extensively used in the food and drink industry; yeast proteases are used extensively in bread baking and beer manufacturing, bacterial and fungal proteases are important in the development of coffee and cocoa and microbial rennets are being increasingly used in the dairy industry (Rao et. al., 1998). Thus the detailed study of keratinases has become significant when the environmental safety is considered and also in the industrial point of view of the enzyme.

Conclusion

The physical and chemical methods of waste feather processing require considerable energy investments. Also, the poor assimilability of amino acids and peptides as well as high production costs of these types of processing direct us for alternative safe biological methods. The application of microbial keratinases could be employed in this current scenario. The keratinolytic microorganisms and technologies developed for feather degradation not only remove the waste feathers efficiently from the nature but also make the by-products of the process as valuable molecules. Over time the large-scale fermentation of keratin wastes that are produced in high amounts, may provide a solution to the problem of their accumulation which otherwise may lead to protein wastage and environmental pollution.

References

1. Adetunji, C.O., Makanjuola, O.R., Arowora K.A., Afolayan, S.S., Adetunji J.B. (2012) Production and application of keratin-based organic fertilizer from microbially hydrolyzed feathers to cowpea (*Vigna unguiculata*), International Journal of Scientific & Engineering Research 12(3).
2. Alberts, B., Bray D., Lewis J., Raff M., Roberts, K., and Watson, J.D., (1994) Molecular Biology of the Cell, 3rd edition, Garland Press, New York.
3. Alessandro Riffel and Adriano Brandelli (2006) Keratinolytic Bacteria Isolated From Feather Waste, Brazilian Journal of Microbiology 37: 395-399.
4. Alexandre, J.M., Walter, O.B., Renata, G., David, D., JoaoAntonio, P.H. and Carlos, T. (2005) Novel keratinase from *Bacillus subtilis* S14 exhibiting remarkable dehairing capabilities, Appl. Environ. Microbiol. 71: 594-596.
5. Bin Zhang, Zhong-Wei Sun, Dan-Dan Jiang and Tian-Gui Niu (2009) Isolation and purification of alkaline keratinase from *Bacillus sp.*, African Journal of Biotechnology 8: 2598-2603.
6. Bockle, B., Galunsky, B., Muller, R. (1995). Characterization of a keratinolytic serine proteinase from *Streptomyces pactum* DSM, Appl. Environ. Microbiol. 61: 3705-3710.
7. Brandelli, A, Daroit, D. J. and Riffel, A (2010) Biochemical features of microbial keratinases and their production and applications, Appl. Microbiol. Biotechnol. 85: 1735-1750.
8. Brandelli, A. and Thys, R.C.S. (2006) Purification and properties of a keratinolytic metalloprotease from *Microbacterium sp.* J Appl. Microbiol. 101 (6): 1259-1268.
9. Bressolier, P., Letourneau, F., Urdaci, M. and Nerneuil, B. (1999) Purification and characterization of keratinolytic serine proteinases from *Streptomyces albidoflavus.* Appl. Environ. Microbiol. 65: 2570-2576.
10. Brutt, E. H. and Ichida, J. M. (1999) Keratinase produced by *Bacillus licheniformis,* US Patent No.5877.
11. Cao, Z.J., Zhang, Q., Wei, D.K., Chen, L., Wang, J., Zhang, X.Q., Zhou, M.H. (2009) Characterization of a novel Stenotrophomonas isolate with high keratinase activity and purification of the enzyme, J. Ind. Microbiol. Biotech. 36:181-188.
12. Cedrola, S.M.L., de Melo, A.C.N., Mazotto, A.M., Lins, U., Zingali, R.B., Rosado, A.S., Peixoto, R.S. and Vermelho, A.B. (2012) Keratinases and sulfide from *Bacillus subtilis* SLC to recycle feather waste, World J. Microbiol. Biotechnol. 28: 1259–1269.
13. De Toni, C.H., Richter M.F., Chagas, J.R., Henriques, J.A.P. and Termignoni, C. (2002) Purification and characterization of an alkaline serine endopeptidase from a feather-degrading *Xanthomonas maltophilia* strain, Canadian Journal of Microbiology 48 (4): 342–348.
14. Dozie, I. N. S., Okeke, C.N. and Unaeze, N. C. (1994) A thermostable, alkaline-active, keratinolytic proteinase from *Chrysosporium keratinophilum*, World Journal of Microbiology and Biotechnology 10(5): 563–567.
15. El-Naghy, M. A., El-Ktatny, M. S., Fadl-Allah, E. M. and Nazeer, W. W. (1998) Degradation of chicken feathers by *Chrysosporium georgiae*, Mycopathologia 143: 77-84.
16. Filipello Marchisio, V., Fusconi, A. and Querio, F.L. (2000) *Scopulariopsis brevicaulis*: a keratinophilic or a keratinolytic fungus, Mycoses 43: 281–292.
17. Fraser, R.B.D., Mac Rae, T.P., Rogers, G.E. (1972) Keratins- their Composition, Structure and Biosynthesis. Charles C. Thomas, Illinois, 283.
18. Freidrich, A.B. and Antranikian, G. (1996) Keratin Degradation by *Fervidobacterium pennavorans*, a novel thermophilic anaerobic species of the order thermotogales, Appl Environ Microbiol. 62(8): 2875-2882.
19. Gessesse, A., Rajni, H.K., Gashe B.A. and Bo Mattiasson (2002) Novel alkaline proteases from alkaliphilic bacteria grown on chicken feather, Enzyme Microbial. Technol. 6250: 1-6.
20. Giongo, J. L., Lucas F. S., Casarin, F., Heeb, P. and Brandelli, P. (2007) Keratinolytic proteases of *Bacillus sp.* isolated from the Amazon basin showing remarkable dehairing activity, World J Microbiol Biotechnol. 23: 375-382.
21. Gupta, R. and Ramnani, P. (2006) Microbial keratinases and their prospective application: an overview, Appl. Microbiol. Biotechnol. 70(1): 21-33.
22. Han, Y., and Parsons, C. M. (1991) Protein and amino acid quality of feather meals, Poultry Sci 70: 812–822.
23. Harrar, B.S. and Woods, E.F. (1963) Soluble derivatives of feather keratin - Isolation, fractionation and amino acid composition, Biochemistry Journal 92: 8-18.
24. Kainoor, P.P and Naik, G.R. (2010) Production and Characterization of feather degrading keratinase from *Bacillus sps.* JB99, Indian Journal of Biotechnology. 9: 384-390.
25. Kaluzewska, M., Wawrzkiewicz, K., Lobarzewski, J. (1991) Microscopic examination of keratin substrates subjected to the action of the enzymes of *Streptomyces fradiae*, Int .Biodeterior. 27: 11-26.
26. Karshan, M. (1930) The chemistry and staining reactions of keratin, J. Dental Research 10: 181-186.
27. Kim, B.C., Lee, D.W., Lee, H.S., Lee, N.J. and Nam, G.W. (2002) Native-feather degradation by *Fervidobacterium islandicum* AW-1, a newly isolated keratinase producing thermophilic anaerobe, Arch. Microbiol., 178: 538-547.
28. Lee, D.L. and Baden, H.P., (1975) Chemistry and composition of the keratins, J. Dermatol. 14: 161–171.
29. Lee, H., Suh, D.B, Hwang, J.H. and Suh, H.J. (2002) Characterization of a keratinolytic metalloprotease from *Bacillus sp.* SCB-3, Appl. Biochem. Biotechnol. 97(2): 123-33.
30. Letourneau, F., Soussotte, V., Bressollier, P., Branland, P., Verneuil, B. (1998) Keratinolytic activity of *Streptomyces sp.* S.K.1-02: a new isolated strain, Lett. Appl. Microbiol. 26: 77-80.
31. Lin, X., Lee, C. G., Casale, E. S. and Shih, J. C. H. (1995) Purification and characterization of keratinase from a feather-degrading *Bacillus licheni-*

formis strain, Applied and Environmental Microbiology 58: 3271-3275.

32. Lin, X., Lee, C.G., Casale, E.S., Shih, J.C.H. (1992) Purification and characterization of a keratinase from a feather-degrading *Bacillus licheniformis* strain, Appl. Environ. Microbiol. 58: 3271-3275.
33. Lucas, F. S., Broennimann, O., Febbraro, I. and Heeb P. (2003) High diversity among feather-degrading bacteria froma dry meadow soil, Microbial Ecology 45(3): 282–290.
34. Mazotto, A.M., de Melo CAN, Macrae, A, Rosado, A.S., Peixoto, R., Cedrola, S.M.L., Couri, S., Zingali, R.B., Villa, A.L.V., Rabinovitch, L., Chaves, J.Q. and Vermelho, A.B. (2011) Biodegradation of feather waste by extracellular keratinases and gelatinases from *Bacillus sp.* World J. Microbiol. Biotechnol. 27: 1355-1365.
35. Mitsuiki, S., Hui, Z., Matzumoto, D., Sakai, M., Moriayama, Y., Furukawa, K., Kanouchi, H., Oka, T. (2006) Degradation of PrPSc by keratinolytic protease from *Nocardiopsis sp.* TOA -1 Biosci Biotchnol Biochem. 70: 1246-1248.
36. Onifade, A.A., Al-Sane, N.A., Al- Musallan, A.A. and Al-Zarban, S. (1998) Potentials for biotechnological applications of keratin degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as lifestock feed resources. Bioresour. Technol. 66: 1-11.
37. Othetellah, N. H., Wang, J.J., Garlich, J.D. and Shih, J.C.H. (2005) Poult Sci 84: 858-864.
38. Papadopoulos, M.C., El Boushy, A.R., Roodbeen, A.E., Ketelaars, E.H. (1986) Effects of processing time and moisture content on amino acid composition and nitrogen characteristics of feather meal Anim. Feed Sci. Tech. 14: 279-290.
39. Parkinson, G. (1998) Chementator: A higher use for lowly chicken feathers, Chem. Eng. 105: 21.
40. Ping Wang, Qiang Wang, Li Cui, Murong Gao and Xuerong Fan. (2011). The combined use of cutinase, keratinase and protease treatments for wool bio-antifelting, Fibers and Polymers 12: 760- 764.
41. Poole, A.J., Church, J.S., Huson, M.G. (2009) Environmentally sustainable fibers from regenerated protein, Biomacromolecules 10: 1-8.
42. Prakash, P., Jayalakshmi, S., and Sreeramulu, K. (2010) Applied Biochemistry and Biotechnology 1909-1920.
43. Rajesh Banu, J., Kaliappan, S., Rajkumar, M. and Dieter Beck (2006) Treatment of spent wash in anaerobic mesophilic suspended growth reactor (AMSGR). J. Environ. Biol. 27: 111-117.
44. Ramnani, P., Singh, R. and Gupta, R. (2005) Keratinolytic potential of *Bacillus licheniformis* RG1: structural and biochemical mechanism of feather degradation, Can J Microbiol., 51: 191-196.
45. Rao, M.B., Tanksale, A.M., Ghatge, M.S., and Deshpande, V.V. (1998) Molecular and biotechnological aspects of microbial proteases, Microbiology and Molecular Biology Reviews 62: 597-635.
46. Riffel, A., Lucas, F., Heeb, P. and Brandelli, A (2003) Characterization of a new keratinolytic bacterium that completely degrades native feather keratin, Archives of Microbiology 179(4): 258–265.
47. Robert Preston, N.D. (2001) Acne - How To Prevent and Overcome Acne forever. International Institute of Nutritional Research 7: 1-17.
48. Safranek, W. W. and Goos, R. D. (1982) Degradation of wool by saprophytic fungi, Can J Microbiol. 28: 137-140
49. Sangali, S. and Brandelli, A. (2000) Feather keratin hydrolysis by a *Vibrio sp.* strain kr2, Journal of Applied Microbiology 89 (5): 735–743.
50. San-Lang Wang, A., Wan-Ting Hsu, A., Tzu-Wen Liang, A., Yue-Horng Yen, B. and Chuan-Lu Wang, C.A. (2008) Purification and characterization of three novel keratinolytic metalloproteases produced by *Chryseobacterium indologenes* TKU014 in a shrimp shell powder medium, Bioresource Technology 99: 5679–5686.
51. Sarita Agrahari and Neeraj Wadhwa (2010) Degradation of chicken feather a poultry waste product by keratinolytic bacteria isolated from dumping site at Ghazipur poultry processing plant, International Journal of Poultry Science 9 (5): 482-489.
52. Sastry, T.P., Sehgal, P.K.., Gupta, B. and Mahendra Kumar (1986) Solublised keratins as a Novel filler in the retaining of upper leather Leather Science. 33: 345-359.
53. Schmidt, W.F. (1998) Innovative feather utilization strategies, Proc. National Poultry Waste Management Symposium, Auburn University Printing Services, October 19-22: 276-282.
54. Schmidt, W.F. (2002) Microcrystalline keratin: from feathers to composite products, In Wallenberger FT, Weston NE, Ford R, Wool RP, Chawla K. (Eds). Proc. Material Research Symposium. December 2-6 U1.5.1-U1.5.5.
55. Selvam, K. and Vishnupriya, B. (2012) Biochemical and molecular characterization of microbial keratinase and its remarkable applications, IJPBA, 3: 267-275.
56. Singh and Pratibha (2007) Sequential anaerobic and aerobic treatment of pulp and paper mill effluent in pilot scale bioreactor, J. Environ. Biol. 28: 77-82.
57. Sohair, A. M. and Assen, M.H., (1974) Biological and biochemical studies on a keratinolytic thermophilic actinomycete, isolated from Egyptian soil, Zentral bl Bakteriol Abt II., 129: 591-599.
58. Subhasish Saha and Dharumadurai Dhanasekaran (2010) Isolation and screening of keratinolytic actinobacteria form keratin waste dumped soil in Tiruchirappalli and Nammakkal, Tamil Nadu, India, Current Research Journal of Biological Sciences 2: 124-131.
59. Suntornsuk, W. and Suntornsuk, L. (2003) Feather degradation by Bacillus sp FK 46 in submerged cultivation. Bioresour. Technol., 86, 239-243.
60. Takami, H., Satoshi, N., Aono, R., Horikoshi, K. (1992). Degradation of human hair by a thermostable alkaline protease from alkaliphilic B*acillus sp.* No. AH-101, Biosci. Biotechnol. Biochem. 56: 1667-1669.
61. Veslava Matikevicience, Danute Masiliunience and Saulius Grigiskis (2009) Degradation of keratin containing wastes by bacteria with keratinolytic activity, Environment. Technology. Resources 1:284-289.
62. Vigneshwaran, C., Shanmugam, S. and Sathish Kumar, T. (2010) Screening and characterization of keratinase from *Bacillus licheniformis* isolated from Namakkal poultry farm, Researcher 2: 89-96.
63. Wang, X. and Parsons, C.M. (1997) Effect of processing systems on protein quality of feather meal and hair meals. Poultry Sci. 76: 491-496.
64. Ward, W.H., Binkley, C.H., Snell, S.N. (1955) Amino acid composition of normal wools, wool fractions, mohair, feather, and feather fractions, Feather Textile Research Journal 25: 314-325.
65. Weitzman, I. and Summerbell, R.C. (1995) The dermatophytes, Clin Microbiol Rev. 8: 240-259.
66. Williams, C.M., Richter, C.S., Mac Kenzie, J.M., Smith, J.M. and Shih J.C. (1990). Isolation, identification and characterization of a feather-degrading bacteriu,. Appl. Environ. Microbiol. 56: 1509-1515.
67. Yamamura, S., Morita, Y. and Hasan, Q. (2002) Characterization of a new keratin-degrading bacterium isolated from deer fur, Journal of Bioscience and Bioengineering 93(6): 595– 600.
68. Yue, X.Y., Zhang, B., Jiang, D.D., Liu, Y.J., Niu, T.G., (2011) Separation and purification of a keratinase as pesticide against root-knot nematodes, World Journal of Microbiology & Biotechnology 27: 2147-2153.