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Microbial Keratinases and their Applications

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Abstract—Bioremediation of feather is an issue requiring serious attention with burgeoning global poultry production which is dumping this byproduct continuously. In spite of being highly rich in protein content, this by-product is neither profitable nor environmentally friendly due to its extremely slow degradation in nature. A value added use of feather is its conversion to feather meal. Traditional methods of feather meal production using physical and chemical treatments result in destruction of essential amino acids thereby affecting both protein quality as well as digestibility. Keratinases have the ability to degrade the tough insoluble keratin of feather and produce nutritious, cost effective, environmentally benign feather meal for poultry. Due to its robust nature, keratinase enzyme is also gaining prominence as feed supplement, organic fertilizer, Degradation of prion proteins, Production of films, coatings and glues and other applications.

Index Terms— Keratinase, bioremediation, Keratinase treated feather, animal protein, applications, animal feed, organic fertilizer,Prion protein.

1 Introduction

Bioremediation of feather is an issue requiring serious attention with burgeoning global poultry production which is dumping this byproduct continuously. In spite of being highly rich in protein content, this by-product is neither profitable nor environmentally friendly due to its extremely slow degradation in nature. Feather is typically made up of 91% of keratin protein, 8% water and 1% of lipids. Composition and molecular configuration of constituent amino acids ensure the structural rigidity of feather. Effective recycling and utilization of this grossly underutilized waste is of great economic and ecological importance. Slow recycling of feather in nature results in accumulation of feather dumps leading to pollution.

Thus inspite of being protein rich, this by-product is under utilized and is thus wasted. Hence, bioremediation of this high voluminous waste has gained immense prominence. Despite being recalcitrance to common proteases, keratin is found to be attacked by some keratinolytic microorganisms and degraded slowly in nature. Several pathogenic and non-pathogenic keratinolytic species belonging to bacteria, actinomycetes and fungi have been reported, majority of which produce Keratinase [1],[2],[3],[4],[5],[6]. Keratinases belong to the group of proteases that can specifically degrade keratin. Microorganism producing this enzyme as well as crude/purified enzyme have enormous application potential [7],[8].

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2 Applications of Keratinases:

Keratinases have potential application in beam house operations of leather and tanning industry to improve the quality of leather for manufacturing leather products and garments [9],[10]. Keratinases are also promising for bioconversion of keratin rich poultry and other industrial wastes into dietary proteins that can be used as food and feed supplements [11],[12],[13]. Keratinases have been shown to be efficient in inactivation/degradation of pathogenic forms of prions, scrapie protein etc. [14]. Thus keratinase production has both economic and ecological value.

Another natural choice for recycling feather is using it as an organic fertilizer. Soil organic matter, nutrients, and biological activity of microorganisms contribute significantly to ecosystem-level processes that are important for productivity, community structure and fertility in terrestrial ecosystems [15]. Feather meal generated by traditional method has also been tested for organic farming as a semi slow release nitrogen fertilizer [16],[17]. However, this feather meal though improved water retention capacity was inferior in nutrition value compared to other organic fertilizers. Thus for profitable crop production, farmers cannot rely on amendment of traditionally manufactured feather meal as sole source of available nitrogen. KTF could be a less expensive substitute to guano due to its superior nutritive value. Analysis of application potential of KTF as animal feed and organic fertilizer are highly desired. These are important avenues for future research and appear as a promising hope for bioremediation of waste feather along with its utilization as animal feed.

In spite of enormous potential application of keratinase in several sectors with both economic and ecological value, the major limiting factor in the wide scale usage of keratinases is the availability of efficient microorganism and designing cost effective method for production of keratinases in large scale. The development of bioprocesses that can convert huge amounts of feather byproducts into added-value products is recognized as an utmost priority [18],[19],[20]. This is essential and appropriate to meet the growing demand for animal products by an ever increasing world population. The upgrading of the nutritional value of feathers will yield an enhanced value protein feed stuff that can replace the use of soyabean and fish meal in livestock diets. Achieving this economically, would be crucial for development of additional and cheap source of animal protein for livestock feeding. Considering the thermo-energetic cost of conventional processing of feathers against the back drop of their limited nutritional improvement, investigation into alternative technology for recycling of feather waste with prospects for nutritional enhancement, environmental compatibility and bioresources optimization are highly justified [21],[22],[23].

Application of keratinase to feather processing has several benefits. Bioconversion using keratinase results in production of structurally modified feather keratin that is less resistant to attack by other digestive enzymes. Secondly, significant nutritional enrichment of the feather meal may be achieved in addition, due to the addition of microbial protein biomass that serves as complementary additive value. Thirdly, increased levels of feed- grade lysine and other amino acids can be targeted in biologically treated FM by supplementing with additional microorganism that can produce amino acid by fermentation also. Biological conversion of feather into feather meal is thus now seriously considered as a source for production of high value dietary proteins [24].

2.1 AS ANIMAL FEED:

An important emerging application of keratinase is as feed supplement. The robust and broad spectrum activity of the enzyme was observed to significantly contribute to growth performance when used in combination with other enzymes like amylases [25],[26]. Enzyme treated feather can also be employed for commercial extraction of important amino acids like Serine, Cysteine, Proline, Cystine etc.[27]. The crude protein from feather has of high nutrient value and could be used as animal feed for livestock and fish feed in aquaculture. Protein as a feed additive can reduce 10% of dietary protein in feed and saving about $10 per ton of feed. Global production of poultry and pig feed is 200 million tons and it has been estimated that total saving of feed cost is $2 billion [28].

2.2 AS ORGANIC FERTILIZER:

Traditionally part of the feather produced was used by gardeners and farmers. However, as immediate soluble nitrogen of feather is too low (being only approximately 0.5%), the availability of the nutrients for plant growth was very poor. Further because of slow mineralization of native feather in nature, it did not make an effective fertilizer. To improve nitrogen accessibility waste feather was grinded into feather powder and added to soil to promote the microbial conversion into soluble nitrogen compounds that could be absorbed by plants. This process however, had only limited success. Enrichment of keratin wastes has also been considered for improvement as soil fertilizers. Organic supplements from different sources, such as blood meal, hooves, horn, feathers, bones and manure have been evaluated as slow-release nitrogen fertilizers [29], [30]. About 70% of nitrogen was released during the first 30 days in field condition with most of the organic supplements except for chicken feathers where the total nitrogen release occurred between 6 and 7 weeks [31],[32]. The slow release of nitrogen from raw feathers indicated that the soil microorganisms could not easily digest the keratin structure. Though feathers with about 15% of nitrogen can be presumed to have great potential to be used as slow-release nitrogen fertilizer, it has not been possible to use this waste effectively. Hydratable keratin and keratin hydro gels produced by treating human hair with a combination of an oxidizing solution and heat have been used as soil amendments and nutrient sources for bioremediation (United States Patent 6649740).

2.3 PRODUCTION OF FILMS, COATINGS AND GLUES:

Recently there has been an increased interest in the production of biodegradable films, coatings and glues from keratinous waste products like hair, feathers, skin, fur, animal hooves, horns etc. for compostable packaging, agricultural films or edible film applications. Keratin structure is chemically modified and hydrolyzed to produce stable dispersions for such applications. Alternatively controlled hydrolysis of keratin using keratinases could offer an environment friendly technology [33].

2.4 DEGRADATION OF PRION PROTEINS

Prions are proteinaceous particles responsible for fatal neurodegenerative diseases called transmissible spongiform encephalopathies (TSE) that include the dreaded mad cow disease, scrapie, kuru and Creutzfeld–Jakob disease. Infectivity by prions is accompanied by the conversion of harmless PrPc to infectious PrPsc, facilitated by PrPsc itself. Shih and coworkers at BRI have reported that the broadspectrum keratinase PWD1 (Versazyme) is capable of completely degrading prions from brain tissue of bovine spongiform encephalopathy (BSE)- and scrapie infected animals in the presence of detergents and heat treatment. The enzymatic breakdown of prions would most importantly help revive the use of animal meal as feed, which faced much criticism by the European Union despite its high nutritive value due to risk of TSE. It would also prove useful for disinfection of hospital facilities and medical devices, slaughterhouse and equipment clean-up, decontamination of animal products, disposal of mortalities and risk materials, decontaminating medical instruments, decontamination of interchangeable items like contact lenses and dentistry tools[28]

2.5 Elimination of Keratin in Acne

Keratinases produced from Bacillus licheniformis (PWD) and E.Coli has been adapted to the laboratory and cosmetic applications especially acne treatment [34]The primary defect in acne is thought to be that there is too much keratin produced at the exist of the hair shaft. This blocks the surface and does not allow the sebum out. So the development of acne is formed. Keratinase is . included in facial scrubs, and it is useful for treating acne, because dead cells can clog pores and create a favorable environment for acne. Some anti-dandruff shampoos include keratinase because the keratinolytic action allows shampoo to wash away flaky dead cells. It can eliminate warts, calluses and corns. The U.S food and drug administration (FDA) has approved keratinase for treating psoriasis, a condition involving excessive turn over of skin cells and scaly build up [35],[36].

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2.6Pharmaceutical enhancement of the Nail

TREATMENT

The topical therapy of Nail disease is limited by the low permeability of drugs through the nail plate. Hydrolytic action of keratinase on nail plate proteins could increase ungula drug delivery. Keratinolytic enzymes might decrease the barrier properties of the nail plate by hydrolyzing the nail keratins. So the enzymatic disruption of nail plate is translated into enhanced drug penetration, into the nail plate [34].

3 CONCLUSIONS

In view of increasing world population and limited resources, recycling of wastes like keratin which is rich in protein is very important. Bioremediation of this cheap and readily available waste and its reutilization has both ecological and economic value. Analysis of application potential of KTF as animal feed and organic fertilizer was highly encouraging. This study provides important leads for future research and opens up new avenues for utilization of bioremediated feather which is highly relevant to the current demands.

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5 REFERENCES

[1] R. A. Young, and R. E. Smith, " Degradation of feather keratin by culture filtrates of Streptomyces fradiae", Canadian Journal of Microbiology. 21: pp.583-586, 1975.

[2] R. K. S.Kushwaha,"The in vitro degradation of peacock feathers by some fungi", Mykosen. 26: pp324–326, 1983

[3] C. M. Williams, C. S .Richter, J. M. Mackenzie, and J. C. H. Shih, Isolation, identification, and characterization of a feather – degrading bacterium. Applied and Environmental Microbiology. 56: pp.1509-1515, 1990.

[4] K. Atalo, and B. A. Gashe," Protease production by a thermophilic Bacillus species (P-001A) which degrades various kinds of fibrous proteins", Biotechnology Letters. 15: pp.1151–1156,1993.

[5] B. Bockle, B. Galunsky, and R. Muller," Characterization of a keratinolytic serine proteinase from Streptomyces pactum DSM 40530", Applied and Environmental Microbiology. 61: pp.3705-3710. 1995.

[6] Santos, R. M. D. B., Firmino, A. A. P., de Sa, C. M. and Felix, C. R. 1996. Keratinolytic activity of Aspergillus fumigatus Fresenius. Current Microbiology. 33: 364-370.

[7] P.Ramnani, R. Singh, and R.Gupta," Optimization of medium composition for keratinase production on feather by Bacillus licheniformis RG1 using statistical methods involving response surface methodology", Biotechnology and Applied Biochemistry. 40: pp.191- 196. 2004.

[8] P.Ramnani, R. Singh, and R.Gupta, "Keratinolytic potential of Bacillus licheniformis RG1: structural and biochemical mechanism of feather degradation. Canadian Journal of Microbiology" 51:pp. 191-196, 2005.

[9] Puvanakrishnan and S. C. Dhar, "Enzyme Technology in Beam hours a practice. Central Leather Research Institute, Chennai, India", pp. 80-158,1988.

[10] J. D. Allpress, G.Mountain and P. C. Gowland, "Production, purification and characterization of an extracellular keratinase from Lysobacter NCIMB 9497". Letters of Applied Microbiology, 34: pp.337-342, 2002.

[11] A. Grazziotin, F. A. Pimental, E. V. de Jong, and A. Brandelli," Nutritional improvement of feather protein by treatment with microbial Keratinase", Animal Feed Sciences and Technology. 126: pp.135-144, 2006.

[12] A Riffel, F. Lucas, P. Heeb, and A. Brandelli, "Characterization of a new keratinolytic bacterium that completely degrades native feather keratin", Archives of Microbiology. 179: 258-265. 2003.

[13] J. C. H.Shih," Purified Bacillus licheniformis PWD-1 keratinase". (U. S. Patents – 5, 171, 682),1992.

[14] M.Yoshioka, T. Miwa, H .Horii, M.Takata, T. Yokoyama, and K.Nishizawa, "Characterization of a proteolytic enzyme derived from a Bacillus strain that effectively degrades prion protein. Journal of Applied Microbiology", 102:pp. 509–515. 2007.

[15] F. J. Stevenson, Humus chemistry: Genesis, composition, reactions. Wiley-Interscience, New York. 512, 1994.

[16] J. M. Choi, and P. V. Nelson, "Developing a slow-release nitrogen fertilizer from organic sources. II. Using poultry feathers. Journal of American Horticultural Society", 121: pp.634-638. 1996.

[17] A. Hadas, and L. Kautsky, "Feather meal, a semi-slow-release nitrogen fertilizer for organic farming", Fertilizer Research. 38: pp.165-170, 1994.

[18] T. I.Zaghloul, M. A. Haroun, K. El-Gayar, and A. Abedalal," Recycling of keratin-containing materials (chicken feather) through genetically engineered bacteria", Polymer–Plastics Technology and Engineering. 43:pp.1589–1599, 2004.

[19] A. Bertsch, and N. Coello, "A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. Bioresource Technology", 96:pp. 1703–1708, 2005.

[20] A. Grazziotin, F. A., Pimentel, S. Sangalide, E. V. Jong, and A. Brandelli, "Production of feather protein hydrolysate by keratinolytic bacterium Vibrio sp. kr2. Bioresource Technology", 98: pp.3172-5. 2007.

[21] A. A. Onifade, N. A. Al-Sane, A. A. Al-Musallam, and S. Al-Zarban, "A review: potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources", Bioresource Technology. 66: pp.1-11, 1998.

[22] Y. Suzuki, Y. Tsujimoto, H. Matsui, and K. Watanabe," Decomposition of extremely hard-to-degrade animal proteins by thermophilic bacteria", Journal of Bioscience and Bioengineering. 102: pp.73–81,2006.

[23] M. Yoshioka, T. Miwa, H., Horii, M.Takata, T. Yokoyama, and K. Nishizawa, "Characterization of a proteolytic enzyme derived from a Bacillus strain that effectively degrades prion protein. Journal of Applied Microbiology",102:pp. 509–515. 2007.

[24] H. Burrows, M. Hurby, D. Hung, and O. Adeola," Addition of enzymes to corn-soy diets for ducks: a performance and digestibility study",Poultry Science. 81: 29-34. 2002.

[25] A., Grazziotin, F. A., Pimental, E. V. de Jong, and A. Brandelli, "Nutritional improvement of feather protein by treatment with microbial Keratinase", Animal Feed Sciences and Technology. 126: pp.135-144, 2006.

[26] M. W. Greenwood, C. A. Fritts, and P. W. Waldroup, "Utilization of enzyme 1502 in cornsoyabean meal diets with and without antibiotics", Poultry Science. 81: 25. 2002.

[27] R. Gupta, and P. Ramnani, "Microbial keratinases and their prospective applications:an overview", Applied Microbiology and Biotechnology. 70: pp.21-33, 2006.

[28] K. Selvam and B .Vishnupriya "Biochemical and Molecular Characterization of Microbial Keratinase and Its Remarkable Applications", International Journal of Pharmaceutical & Biological Archives; 3(2):pp267-275, 2012.

[29] Hadas, A. and Kautsky, L. 1994. Feather meal, a semi-slow-release nitrogen fertilizer for organic farming. Fertilizer Research. 38: 165-170.

[30] J. M. Choi, and P. V. Nelson, "Developing a slow-release nitrogen fertilizer from organic sources. II. Using poultry feathers", Journal of American Horticultural Society. 121:pp. 634-638,1996.

[31] A. Hadas, and L. Kautsky, "Hydratable form of keratin for use as a soil amendment", United States Patent 6649740.

[32] C. M. Williams, C. S. J. M.RichterMackenzie and J. C. H. Shih," Isolation, identification, and characterization of a feather – degrading bacterium", Applied and Environmental Microbiology. 56: pp.1509-1515. 1990.

[33] P.Ramnani, R. Singh, and R.Gupta, "Keratinolytic potential of Bacillus licheniformis RG1: structural and biochemical mechanism of feather degradation. Canadian Journal of Microbiology" 51:pp. 191-196, 2005.

[34] Subhasish Saha and Dharumadurai Dhanasekaran," Isolation and Screening of Keratinolytic Actinobacteria form Keratin Waste Dumped Soil in Tiruchirappalli and Nammakkal, Tamil Nadu", Indi. Current Research Journal of Biological Sciences., 2: pp.124-131, 2010.

[35] Veslava Matikevicience, Danute Masiliunience and Saulius Grigiskis," Degradation of keratin containing wastes by Bacteria with keratinolytic activity", Environment Technology Resources, 1:pp.284-289, 2009.

[36] C, S. Vigneshwaran Shanmugam, and T. Sathish Kumar" Screening and Characterization of Keratinase from Bacillus licheniformis Isolated From Namakkalpoultry Farm",Researcher, 2: pp.89-96, 2010;