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Profiling Protease With Broad Substrate Affinity And Catalytic Diverse From Poultry Industry; In Perspective.

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ABSTRACT

The enzymes are integral part of research, healthcare, agriculture and commercial industries with their increasing demand for extended applications. Notable, the enzymes are unique biomolecule with their role in catalysing biochemical reactions vital for physiology and commercial applications as well. The role of enzymes in the commercial applications has grown tremendously during last few decades due to many reasons such as specificity towards substrate, economical and most important eco-friendly. The increasing demand of this biocatalyst posed a challenge to the researcher not only profile new enzymes but also novel sources as well. This led to search of novel sources for various enzymes and profiling of these enzymes in the different industrial applications. Among the six class of enzymes protease represent one of most diverse and useful enzymes for proteolytic process in physiology and industry. Agro-industrial based keratinous wastes are one of major grown challenge and lack of effective eco-friendly mechanism to recycle is required immediately. In such scenario, keratinase enzymes are eco-friendly option not only to recycle the waste but also produce commercially useful products. Keratinase is an enzyme that breaks down keratin, a rigid protein found in hair, wool, and feathers, into amino acids. Keratinase can be used in many industries for recycling of waste. Hence, there is growing demand of the enzyme and novel sources as well. In this study a novel source as poultry was examine and profiled for the novel source of proteases and application in the industry.

Keywords: Poultry, Protease, Keratinase, proteolytic enzymes, Waste recycling and waste management.

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Enzyme and enzyme action/Mechanism

The proteins that catalyse life are called enzymes. Families and superfamilies are groups of enzymes that share a common ancestor based on similarities in sequence and structure. The Enzyme Commission manually classifies the molecular function of enzymes as their capacity to catalyse biological events, and reliable methods for statistically comparing catalytic reactions are only now starting to emerge (1). Enzymes are bio molecular with immense property to catalyse the biochemical reactions in the biological milieu and industrial scale as well. Enzymes are unique to provide the products from a biochemical reaction very specific to a substrate. In general, a chemical and biochemical reaction without enzyme requires longer time and often did not get completed. The use of enzyme not only speeds up a biochemical reaction but also provide a time scale for the yield of products (2). Oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases are the six primary types of enzymes. Each category catalyzes a wide variety of unique reactions within their own category while carrying out a general sort of reaction. Certain enzymes, referred to as apoenzymes, are dormant until they attach to a cofactor, which causes the enzyme to become active. Metal ions (like zinc) or organic substances that bind to the enzyme covalently or noncovalently can both be considered cofactors (3). A holoenzyme is the compound of an apoenzyme and a cofactor. Proteins called enzymes are made up of one or more polypeptide chains connecting amino acids. The main structure of a polypeptide chain is this arrangement of amino acids. subsequently establishes the enzyme's three-dimensional structure, which includes the active site's form (1, 4).

Enzyme and their Sources

Since enzymes catalyse and coordinate the intricate events of cellular metabolism, they are vital parts of all living things, including microbes, plants, and animals. Most enzymes used in commerce up to the 1970s were from plant and animal sources. Enzymes derived from plants and animals were favoured because they were thought to be free of the toxicity and contamination issues that were linked to enzymes of microbial origin (5). At the time, bulk enzymes were often only utilized in the food processing sector. But as demand increased and fermentation technology advanced, microbial enzymes' competitive price was acknowledged, leading to their increased utilization. Compared with enzymes from plant and animal sources, microbial enzymes have economic, technical and ethical advantages. In contrast to microbes, which can typically be produced and extracted at the same site, animal and plant sources typically require transportation to the extraction facility (3). Furthermore, commercially significant plant and animal enzymes are frequently found in a single organ or tissue, making the remainder effectively a waste product that must be disposed of. Lastly, while microbial enzymes are not affected by these issues, enzymes derived from plant and animal sources exhibit significant yield variation and may only be available during specific seasons (6).

Microbial enzymes

Since microbial enzymes are known to be essential metabolic catalysts, they are used in a wide range of sectors and applications. Industrial enzymes have several industrial commercial applications and a very broad end-use market. Enzymes are used in the production of more than 500 industrial items (7). The increasing need for sustainable solutions is driving a steady increase in the demand for industrial enzymes. One of the biggest and most practical sources of many enzymes has been and still is microbes. Low catalytic efficiency, lack of enantiomeric specificity for chiral synthesis, high temperature, low pH, and high pressure are some of the drawbacks of many industrial processes, including chemical synthesis for the creation of chemicals and pharmaceuticals (8). Additionally, using organic solvents produces pollutants and organic waste. Because enzymes operate in mild reaction conditions (temperature, pH, and atmospheric conditions), do not require the protection of substrate functional groups, have a long half-life, have a high degree of stereo-selectivity, produce reaction products that are stereo- and regiochemically defined at an acceleration of 105 to 108 times, and work on unnatural substrates, they are more beneficial for these applications. In addition, enzymes can be chemically and genetically altered to improve their stability, substrate specificity, and particular activity (9). However, this issue can be resolved using a variety of strategies, including the utilization of entire cells and cofactor recycling. Enzymes or complete microbial cell catalysts are used in about 150 industrial processes (10).

Application of microbial enzymes

Although they have long been used extensively in the food and beverage sectors, biocatalysts are now finding new uses in a variety of disciplines, including industrial chemistry. When used as catalysts for any of the processes, biocatalysis uses whole microbial cells, cell extracts, pure enzymes, immobilized cells, or immobilized enzymes. For processes of industrial, medicinal, and biotechnological importance, enzymes are crucial (11). By 2010, the industrial enzymes market was worth $3.3 billion, and by 2015, it is projected to be around 4.4 billion. Among them, technical enzymes are commonly employed as bulk enzymes in the pulp and paper, detergent, textile, and biofuels sectors, among others. The two products with the highest sales figures are leather and bioethanol. Revenues from technical enzymes were close to $1.2 billion in 2011, and they are predicted to grow to $1.5 billion in 2015 and $1.7 billion in 2016 (12). The market for biofuels (bioethanol) is anticipated to have the largest sales. By 2015, it is anticipated that the use of enzymes in food and drink will total $1.3 billion.

More than 60% of the enzyme market worldwide is made up of proteases. They are employed in the manufacturing of agrochemicals, foods, detergents, leather, silk, and medications. They make up around a quarter of all enzyme sales worldwide in laundry detergents (13). Enzymes are also being utilized more in the textile sector to create cleaner procedures, use fewer raw materials, and generate less waste. The most recent commercial developments are the use of laccases for textile bleaching and decolorization of textile effluents, and cellulases for denim finishing. The use of lipases, xylanases, and laccases in the pulp industry to remove pitch—hydrophobic wood components mostly triglycerides and waxes—is another increasingly significant application of enzymes (14). Nippon Paper Industries uses a Candida rugosa lipase to eliminate up to 90% of these substances. Enzymes have been successfully used in place of chemicals in the leather processing industry to improve leather quality and lessen pollution in the environment. Currently, this industry uses alkaline lipases from Bacillus strains that develop in extremely alkaline environments in conjunction with other alkaline or neutral proteases (15). Despite their long history of widespread use in textile applications, cellulases are currently receiving more attention in the enzyme market due to their capacity to break down lignocellulosic feedstocks.

Poultry Industry as Source of enzyme

Globally, livestock output is rising quickly due to factors like population expansion, rising affluence, and dietary and lifestyle changes. In addition to the continued growth in bovine and pork meat output, the UN Food and Agriculture Organization (FAO) projects that the world's poultry production will reach over 24.8 billion animals annually by 2030 and 37.0 billion by 2050 (1.7 kg carcass weight/animal) (9, 11). The cyclic emptying of the various gastrointestinal tract regions causes rapid temporal changes in the microbiota present in frequently used non-invasive samples, such as excreta and cloacal swabs. In the chicken intestine, Firmicutes is the most prevalent phylum overall. The main bacterial taxa present in the crop, gizzard, duodenum, and ileum are Lactobacillus, while Bifidobacterium and Enterobacter are also frequently seen in the crop (13). There have also been reports of Escherichia and Enterococcus (phylum Proteobacteria) and Clostridium, Ruminococcus, Streptococcus, Candidatus, and Arthromitus (phylum Firmicutes) in the ileum (16).

Keratinase

Keratinases are essential for managing keratin waste because they are the only proteolytic enzymes that can break down stubborn insoluble proteins (17). In recent decades, scientists have concentrated on identifying keratinase producers as well as generating and describing keratinases. The feed, fertilizer, leathering, detergent, cosmetic, and medical industries have all investigated the possible uses of keratinases (18). Wool, feathers, and hair are examples of very stiff keratin wastes that can be specially hydrolyzed by the special enzyme keratinase. These significant agroindustry byproducts are produced annually in vast amounts throughout the world, but because of their tight structure and high disulfide-bond content, they are challenging to dispose of. Many different types of bacteria, including eukaryotic and prokaryotic ones, can manufacture keratinase (19). Decomposing feathers, poultry manure, and slaughterhouse waste are among the keratin-rich habitats from which most of these microbes are isolated. Another significant source of strains that produce keratinase is the marine environment. Using samples of damaged feathers from saltwater shorelines, Herzog et al. found 50 distinct bacterial species that could break down keratin (20). Two keratinases were discovered to be

produced by Bacillus amyloliquefaciens S13, which was isolated from maritime brown algae. Researchers have discovered in recent years that keratinase-producing strains can also be found in animals (21).

Enzyme insight

The only class of proteases with a broad pH and temperature range that can completely break down complex and resistant proteins are keratinases (EC 3.4.21). The capacity of keratinases to attach to complex and insoluble substrates (hair, azokeratin, nails, horns, stratum corneum, collagen, elastin, feathers, wool, and silk) sets them apart from other proteases (22, 23). The degree of keratin hydrolysis is known to increase with adsorption capacity, even though the mechanism of enzyme adsorption is still poorly understood. Following enzyme binding and disulfide link breaking, keratin undergoes a conformational shift that makes many sites available for the enzymes' hydrolytic action (24, 25). Keratinases are classified as serine-and metalloproteases or serine metalloproteases based on the characteristics of their active site (26).

A class of proteases known as keratinases can break down keratin by cleaving its peptide links (27). Despite being serine and metalloproteases that may break peptide bonds in peptide chains, the discovered keratinases can detect hydrophobic substrates and have an impact on disulfide bonds. Most keratinases need additional enzymes to break down the disulfide, and keratin degradation involves two steps: peptide degradation and keratin peptide release. Reducing agents or disulfide reductases can accelerate a reduction reaction (28). Other enzymes or substances are required to influence the disulfide bonds and lessen the forces for keratin packing to make proteins accessible to the proteases, since most keratinases are proteases that break peptide bonds (29). As a result, keratin degradation involves at least two processes, including proteolysis and disulfide bond breaking (30). It has been shown that bacteria and fungi break down keratins in rather different ways, with the exception that fungi also use mechanical destruction (31).

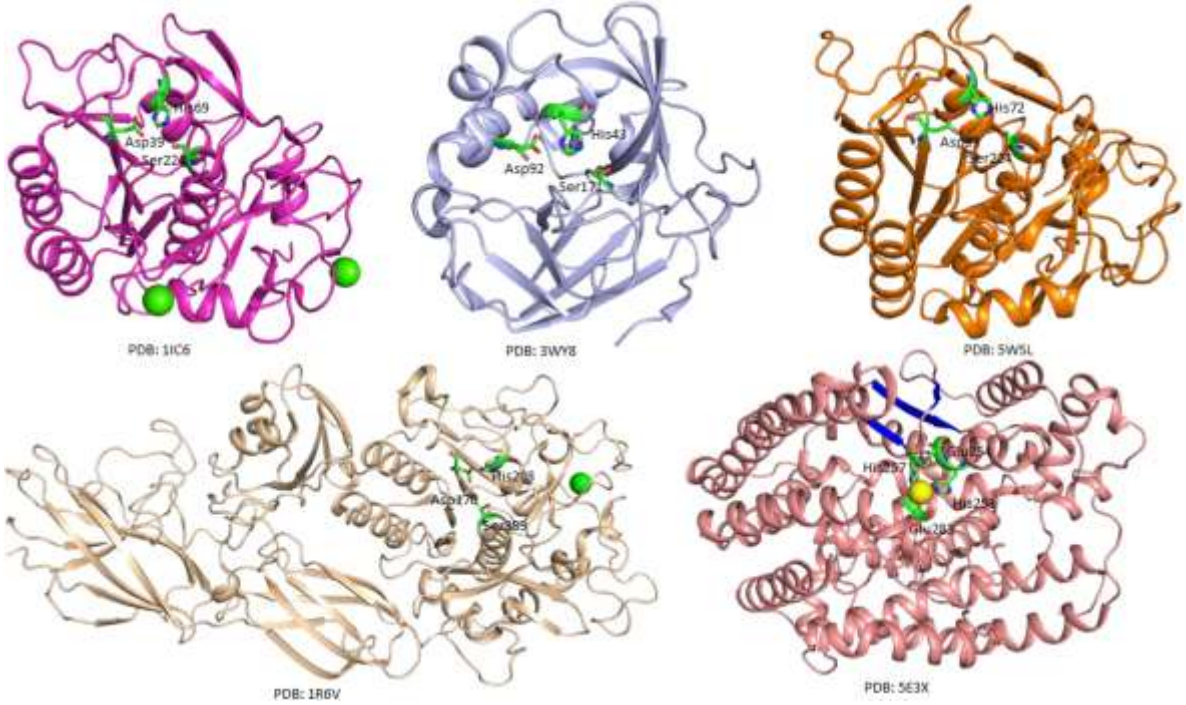

Figure 1: Representation of microbial keratinase enzyme structure and active forms (31).

Several conserved residues compose the active sites of keratinases, which are serine and metalloproteases. Several keratinases' crystal structures show the structural underpinnings of their action and offer guidance for creating more stable and effective enzymes for use in industry. It is also possible to create homology models of different keratinases using these structures as a template (32). The molecular weights of keratinase range from 20 to 130 kDa, according to the sequences that have been entered into databases and the discovered enzymes. Since different enzymes have varying preferences for pH, temperature, and substrates, there are many potential ideal conditions for enzymatic activity (33). Getting the amino acid sequence of the recently discovered keratinases is a crucial endeavour since the

classification of keratinases based on their amino acid sequence offers a distinct and transparent perspective on their mechanism and function. Most of the research examined the ability of keratinases to break peptide bonds, even though the impact of keratinase producers on disulfide bond breaking should be quantified (34, 35). The enzymatic assay used several substrates to confirm the capacity to break down keratin.

Application of Keratinase enzyme

Keratinases are produced by a variety of bacteria. They are effective at breaking the disulfide bonds in keratin, which makes it easier to convert keratin from complex to simpler forms (36). Keratinases are proteolytic enzymes that have a wide range of applications, including

Leather industry: Keratinases are used to gently remove hair from animal hides without the use of strong chemicals. One of the world's oldest and fastest-growing businesses, the leather sector is crucial to the modern economy. However, because leather processing involves the use of harmful compounds that have a negative impact on both the environment and industrial plant workers, it is regarded as one of the largest producers of pollution in the world (37).

Textile industry: Keratinases are used in the finishing treatment of textiles. Eighty-nine percent of the proteolytic enzymes utilized in the detergent industry are alkaline proteases. In addition to exhibiting stability and activity at higher pH levels and temperatures, enzymes that are suitable for use in detergents must be compatible with other ingredients in the washing agents. One enzyme that may be used in the laundry industry to remove compound stains without compromising the texture, fibers, or strength of clothing is the alkaline keratinase from Paenibacillus woosongensis TKB2 (38).

Waste management: Keratinases are used to convert keratin-rich wastes, such as feathers from poultry, into value-added products. Other uses for keratinases include the bioaugmentation of composting keratin-rich waste, the alteration of the fundamental structure of fibers in wool or silk, the production of edible bird nests, and other cosmetic goods. Pearl bleaching is one of the non-traditional uses (39). Organic contaminants such free cells, mucus cells, and necrotic tissue may be present in the mounting layer during the bead formation process. As a result, beads must be improved in quality before being sold. Gentle bleaches, such as hydrogen peroxide, are commonly used to treat pearls. These bleaches can brighten the pearls gently, but they can also cause color irregularities and counterbalance their coloring (16).

Pharmaceutical and biomedical industries: Keratinases are used in these industries due to their ability to cleave peptides. In the cosmetics business, keratinases are also used to treat psoriasis, calluses, keratinized and dry skin, acne, and other conditions (320. The pharmaceutical industry uses keratinases primarily to improve the way fungicidal medications pass through the surface of keratinous nails. Fungal infections are the primary cause of nail problems, which range from painful symptoms like nail dystrophy to relatively benign conditions like pigmentation (21). Onychomycosis, a fungal infection of the nails, is very difficult to treat and is typically treated with repeated monthly injections of corticosteroids and long-term use of antifungal medications, which can have several negative effects, including liver damage and rashes (39).

Detergent formulation: Keratinases are used in the formulation of detergents. Purified from this strain, an alkaline β-keratinase (Brevicarnase) with a molecular mass of 83.2 kDa exhibited peak activity at pH 12.5 and 45 °C, respectively. In relation to keratin, the Km and Vmax values of β-keratinase were found to be 0.3 mg ml−1 and 4.5 μmol min−1 mg−1, respectively (40). At a concentration of 0.1%, the Brevicarnase showed good thermo-stability and stability in the presence of oxidizing and bleaching agents, anionic and non-ionic surfactants, EDTA, and compatibility with the tested commercial laundry detergents. When tested on goat skin, the pure β-keratinase showed dehairing properties but no collagen-degrading activity (18).

Animal nutrients and protein supplements: Keratinases are used in the preparation of animal nutrients and protein supplements. Keratin, a complex protein present in hard tissues such as hair, feathers, nails, and wool in both humans and animals, is broken down by these proteolytic enzymes. To make keratin appropriate for use in animal feed and protein supplements, keratinases break it down into simpler forms (41).

Feather meal processing: Keratinases are used in the processing of feather meal for feed and fertilizer. Feathers, which make up 7–10% of chicken mass, are the primary waste product in the poultry industry. Approximately 8.5 billion tonnes of poultry feathers were produced globally in 2012. About 90% of feathers are made of keratin, with β-keratin making up the bulk (42). They also contain significant amounts of proline, glutamic acid, serine, and tiny amounts of histidine, lysine, and methionine. One of the largest waste byproducts of the chicken business and a significant contributor to environmental contamination is the several million tonnes of feathers produced annually. Making feather meal, which can be utilized as an ingredient in bioplastics, a raw material for the manufacturing of biodiesel, or animal feed, is one way to value feather waste (43).

Sewage system obstructions: Keratinases are used to clean and treat obstructions in sewage systems. Keratinases have been investigated for the treatment of highly contaminated industrial wastewaters, notably molasses wastewater (MWW) from sugar factories, which contains melanoidins and other dangerous chemicals (44). Conventional secondary aerobic and anaerobic biological treatments are ineffective at decolorizing MWW because of high concentrations of contaminants and co-pollutants, oxidation resistance, and frequently strong antimicrobial effects. In treated MWW (TMWW), melanoidins, phenols, and caramel are the main colorants; the first category is the most prevalent (07).

Wool cleaning: Keratinases are used to clean wool. Wool, feathers, and hair are examples of very stiff keratin wastes that can be specially hydrolysed by the special enzyme keratinase. These significant agroindustry byproducts are produced annually in vast amounts throughout the world, but because of their tight structure and high disulfide-bond content, they are challenging to dispose of (45).

Challenges and limitations

The key challenge with the keratinase enzyme is lack of high capacity and yielding source. The microbes producing keratinase are yet of characterize where poultry industry is one. There are several issues with the current methods used to characterize keratinases. The assays are not standardized in the literature in terms of reaction conditions and substrates. The most common method used to measure keratinase activity is a colorimetric assay that uses the commercially available derivative of wool, keratin azure or azokeratin (sulfanilic acid-azokeratin (16). However, batch variability and the fact that the chromogenic agents are only bound to the outer portion of the substrate compromises reproducibility. Quantification of the soluble peptides generated by hydrolysis of keratin has also been used to determine the effectiveness of keratinases on keratin substrates (46).

Future perspective

A sustainable and promising scope of microbes in production of keratinase is under investigated. Because of its special and effective hydrolysis capacity, keratinase has demonstrated significant importance and application potential in the biodegradation and recycling of keratin waste. Nevertheless, the enzyme's intrinsic instability restricts its usefulness. Conventional keratin breakdown techniques, including mechanical grinding, composting, incineration, and burial, release harmful gasses that are detrimental to both land and marine life (47). The nutritional value of the amino acids produced by keratin breakdown is decreased by the expensive and energy-intensive chemical treatment of keratin wastes (acid/alkaline hydrolysis or treatment with oxidizing/reducing agents). By comparing the target sequence (predicted structure) to fully defined enzymes, rational protein design can efficiently predict and alter the residues that give rise to the enzyme's substrate and co-factor binding sites, thermostability, and other particular functional features. One logical method in protein engineering for altering the substrate selectivity of enzymes is site-directed mutagenesis.

CONCLUSION

Poultry is key source of various microbes both pathogenic and non-pathogenic. Among these the useful microbes are being investigated for potential applications in various commercial industry. It is commonly known that one of the main factors propelling the growth of bio-industrial sectors can be the utilization of natural resources to identify possible microorganisms. We screened environmental samples from the northeastern part of India, which is regarded as one of the world's greatest biodiversity zones, for bacteria that produce alkaline protease (β-keratinase). In this study, we screen a soil sample from Assam, North-east India, and isolate a promising bacterium, Brevibacillus sp. AS-S10-II, that produces

alkaline β-keratinase. As far as we are aware, this is the first report on the synthesis and purification of the Brevibacillus sp. β-keratinase enzyme. Progressive research is ongoing to identifying and profiling potential enzymes from the poultry industry.

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REFERENCES

[1] Qiu J, Wilkens C, Barrett K, Meyer AS. Microbial enzymes catalyzing keratin degradation: Classification, structure, function. Biotechnol Adv. 2020 Nov 15;44:107607. doi: 10.1016/j.biotechadv.2020.107607.

[2] Thadiyan V, Sharma V, Gupta R. Keratinase and its diverse applications. 3 Biotech. 2025 Jun;15(6):151. doi: 10.1007/s13205-025-04319-0.

[3] Gopinath SC, Anbu P, Lakshmipriya T, Tang TH, Chen Y, Hashim U, Ruslinda AR, Arshad MK. Biotechnological Aspects and Perspective of Microbial Keratinase Production. Biomed Res Int. 2015;2015:140726. doi: 10.1155/2015/140726.

[4] Sypka M, Jodłowska I, Białkowska AM. Keratinases as Versatile Enzymatic Tools for Sustainable Development. Biomolecules. 2021 Dec 18;11(12):1900. doi: 10.3390/biom11121900.

[5] Cai CG, Chen JS, Qi JJ, Yin Y, Zheng XD. Purification and characterization of keratinase from a new Bacillus subtilis strain. J Zhejiang Univ Sci B. 2008 Sep;9(9):713-20. doi: 10.1631/jzus.B0820128.

[6] Nnolim NE, Udenigwe CC, Okoh AI, Nwodo UU. Microbial Keratinase: Next Generation Green Catalyst and Prospective Applications. Front Microbiol. 2020 Dec 18;11:580164. doi: 10.3389/fmicb.2020.580164.

[7] Moktip T, Salaipeth L, Cope AE, Taherzadeh MJ, Watanabe T, Phitsuwan P. Current Understanding of Feather Keratin and Keratinase and Their Applications in Biotechnology. Biochem Res Int. 2025 Apr 22;2025:6619273. doi: 10.1155/bri/6619273.

[8] Qiu J, Barrett K, Wilkens C, Meyer AS. Bioinformatics based discovery of new keratinases in protease family M36. N Biotechnol. 2022 May 25;68:19-27. doi: 10.1016/j.nbt.2022.01.004.

[9] Li Q. Structure, Application, and Biochemistry of Microbial Keratinases. Front Microbiol. 2021 Jun 23;12:674345. doi: 10.3389/fmicb.2021.674345.

[10] de Menezes CLA, Santos RDC, Santos MV, Boscolo M, da Silva R, Gomes E, da Silva RR. Industrial sustainability of microbial keratinases: production and potential applications. World J Microbiol Biotechnol. 2021 Apr 17;37(5):86. doi: 10.1007/s11274-021-03052-z.

[11] Alwakeel SS, Ameen F, Al Gwaiz H, Sonbol H, Alghamdi S, Moharram AM, Al-Bedak OA. Keratinases Produced by *Aspergillus stelliformis*, *Aspergillus sydowii,* and *Fusarium brachygibbosum* Isolated from Human Hair: Yield and Activity. J Fungi (Basel). 2021 Jun 10;7(6):471. doi: 10.3390/jof7060471..

[12] Khan A, Mehmood K, Nadhman A, Khan SU, Shah AA, Shah Z. Microbial production of keratinase from *Bacillus velezensis* strain MAMA: A novel enzyme for eco-friendly degradation of keratin waste. Heliyon. 2024 Jun 4;10(12):e32338. doi: 10.1016/j.heliyon.2024.e32338.

[13] Mpaka L, Nnolim NE, Nwodo UU. Microbial Keratinolysis: Eco-Friendly Valorisation of Keratinous Waste into Functional Peptides. Microorganisms. 2025 Sep 27;13(10):2270. doi: 10.3390/microorganisms13102270.

[14] Vidmar B, Vodovnik M. Microbial Keratinases: Enzymes with Promising Biotechnological Applications. Food Technol Biotechnol. 2018 Sep;56(3):312-328. doi: 10.17113/ftb.56.03.18.5658.

[15] Bian F, He H, Chen G, Yue S, Zhu Y, Zhang X, Xie BB. A Highly Active Keratinase from *Bacillus* sp. FJ-3-16 for Sustainable Feather Waste Valorization and Eco-Friendly Industrial Applications. Biomolecules. 2025 Sep 29;15(10):1389. doi: 10.3390/biom15101389.

[16] Nnolim NE, Nwodo UU. Microbial keratinase and the bio-economy: a three-decade meta-analysis of research exploit. AMB Express. 2021 Jan 7;11(1):12. doi: 10.1186/s13568-020-01155-8.

[17] Nnolim NE, Mpaka L, Okoh AI, Nwodo UU. Biochemical and Molecular Characterization of a Thermostable Alkaline Metallo-Keratinase from *Bacillus* sp. Nnolim-K1. Microorganisms. 2020 Aug 27;8(9):1304. doi: 10.3390/microorganisms8091304.

[18] Nnolim N.E., et al. Microbial keratinase: next generation green catalyst and prospective applications. Front. Microbiol. 2020;11 doi: 10.3389/fmicb.2020.580164.

[19] Vidmar B., Vodovnik M. Microbial keratinases: enzymes with promising biotechnological applications. Food Technol. Biotechnol. 2018;56(3):312–328. doi: 10.17113/ftb.56.03.18.5658.
[20] Brandelli A. Bacterial keratinases: useful enzymes for bioprocessing agroindustrial wastes and beyond. Food Bioprocess Technol. 2008;1(2):105–116.
[21] Călin M., et al. Degradation of keratin substrates by keratinolytic fungi. Electron. J. Biotechnol. 2017;28:101–112.
[22] Rios P., et al. Production and characterization of a new detergent-stable keratinase expressed by Pedobacter sp. 3.14.7, a novel Antarctic psychrotolerant keratin-degrading bacterium. J. Genet. Eng. Biotechnol. 2022;20(1):81. doi: 10.1186/s43141-022-00356-x.
[23] Kainoor P., Naik G.R. Production and characterization of feather degrading keratinase from Bacillus sp. JB 99. Indian J. Biotechnol. 2010;9:384–390.
[24] Kumar M.S. Genomic DNA isolation from fungi, algae, plant, bacteria and human blood using CTAB. Int. J. Sci. Res. 2014;3(9)
[25] Wu W.-L., et al. The discovery of novel heat-stable keratinases from Meiothermus taiwanensis WR-220 and other extremophiles. Sci. Rep. 2017;7(1):4658. doi: 10.1038/s41598-017-04723-4.
[26] Lowry O.H., et al. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951;193(1):265–275.
[27] Brunelle J.L., Green R. One-dimensional SDS-polyacrylamide gel electrophoresis (1D SDS-PAGE) Methods Enzymol. 2014;541:151–159. doi: 10.1016/B978-0-12-420119-4.00012-4.
[28] Balaji S., et al. Purification and characterization of an extracellular keratinase from a hornmeal-degrading Bacillus subtilis MTCC (9102) World J. Microbiol. Biotechnol. 2008;24(11):2741–2745.
[29] Sharma I., et al. Parametrically optimized feather degradation by Bacillus velezensis NCIM 5802 and delineation of keratin hydrolysis by multi-scale analysis for poultry waste management. Sci. Rep. 2022;12(1) doi: 10.1038/s41598-022-21351-9.
[30] Brandelli A., Daroit D.J., Riffel A. Biochemical features of microbial keratinases and their production and applications. Appl. Microbiol. Biotechnol. 2010;85(6):1735–1750. doi: 10.1007/s00253-009-2398-5.
[31] Li Q. Structure, Application, and Biochemistry of Microbial Keratinases. Front Microbiol. 2021 Jun 23;12:674345. doi: 10.3389/fmicb.2021.674345. PMID: 34248885; PMCID: PMC8260994.
[32] Abdel-Fattah A.M., et al. Biodegradation of feather waste by keratinase produced from newly isolated Bacillus licheniformis ALW1. J. Genet. Eng. Biotechnol. 2018;16(2):311–318. doi: 10.1016/j.jgeb.2018.05.005.
[33] Cai C.-g., et al. Purification and characterization of keratinase from a new Bacillus subtilis strain. J. Zhejiang Univ. - Sci. B. 2008;9(9):713–720. doi: 10.1631/jzus.B0820128.
[34] Barman N.C., et al. Production, partial optimization and characterization of keratinase enzyme by Arthrobacter sp. NFH5 isolated from soil samples. Amb. Express. 2017;7(1):181. doi: 10.1186/s13568-017-0462-6.
[35] Sanghvi G., et al. A novel alkaline keratinase from Bacillus subtilis DP1 with potential utility in cosmetic formulation. Int. J. Biol. Macromol. 2016;87:256–262. doi: 10.1016/j.ijbiomac.2016.02.067.
[36] Haq I., Akram F. 2019. Striking Applications of Keratinase Enzyme Isolated from Various Natural Sources: A Review; pp. 1–17. 55.
[37] Dlume T., Nnolim N.E., Nwodo U.U. Exiguobacterium acetylicum transformed poultry feathers into amino acids through an extracellular secretion of keratinolytic enzymes. AIMS Bioeng. 2024;11:489–505. doi: 10.3934/bioeng.2024022.
[38] Lange L., Huang Y., Busk P.K. Microbial decomposition of keratin in nature—A new hypothesis of industrial relevance. Appl. Microbiol. Biotechnol. 2016;100:2083–2096. doi: 10.1007/s00253-015-7262-1.
[39] Peng Z., Mao X., Zhang J., Du G., Chen J. Biotransformation of keratin waste to amino acids and active peptides based on cell-free catalysis. Biotechnol. Biofuels. 2020;13:61. doi: 10.1186/s13068-020-01700-4.
[40] Fang Z., Zhang J., Liu B., Du G., Chen J. Biodegradation of wool waste and keratinase production in scale-up fermenter with different strategies by Stenotrophomonas maltophilia BBE11-1. Bioresour. Technol. 2013;140:286–291. doi: 10.1016/j.biortech.2013.04.091.
[41] Kshetri P., Singh P.L., Chanu S.B., Singh T.S., Rajiv C., Tamreihao K., Singh H.N., Chongtham T., Devi A.K., Sharma S.K., et al. Biological activity of peptides isolated from feather keratin waste through microbial and enzymatic hydrolysis. Electron. J. Biotechnol. 2022;60:11–18. doi: 10.1016/j.ejbt.2022.08.001.

[42] Wan M.Y., Dong G., Yang B.Q., Feng H. Identification and characterization of a novel antioxidant peptide from feather keratin hydrolysate. Biotechnol. Lett. 2016;38:643–649. doi: 10.1007/s10529-015-2016-9.

[43] Qin X., Xu X., Guo Y., Shen Q., Liu J., Yang C., Scott E., Bitter H., Zhang C. A sustainable and efficient recycling strategy of feather waste into keratin peptides with antimicrobial activity. Waste Manag. 2022;144:421–430. doi: 10.1016/j.wasman.2022.04.017

[44] Sharma I., Pranaw K., Soni H., Rawat H.K., Kango N. Parametrically optimized feather degradation by Bacillus velezensis NCIM 5802 and delineation of keratin hydrolysis by multi-scale analysis for poultry waste management. Sci. Rep. 2022;12:17118. doi: 10.1038/s41598-022-21351-9.

[45] Zaghloul T.I., Embaby A.M., Elmahdy A.R. Key determinants affecting sheep wool biodegradation directed by a keratinase-producing Bacillus subtilis recombinant strain. Biodegradation. 2011;22:111–128. doi: 10.1007/s10532-010-9381-9

[46] Kshetri P., Roy S.S., Sharma S.K., Singh T.S., Ansari M.A., Prakash N., Ngachan S.V. Transforming chicken feather waste into feather protein hydrolysate using a newly isolated multifaceted keratinolytic bacterium Chryseobacterium sediminis RCM-SSR-7. Waste Biomass Valor. 2019;10:1–11. doi: 10.1007/s12649-017-0037-4.

[47] Hamiche S., et al. Purification and biochemical characterization of two keratinases from Bacillus amyloliquefaciens S13 isolated from marine brown alga Zonaria tournefortii with potential keratin-biodegradation and hide-unhairing activities. Int. J. Biol. Macromol. 2019;122:758–769. doi: 10.1016/j.ijbiomac.2018.10.174.