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Molecular strategies to enhance the keratinase gene expression and its potential implications in poultry feed industry

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ABSTRACT The tons of keratin waste are produced by the poultry and meat industry which is an insoluble and protein-rich material found in hair, feathers, wool, and some epidermal wastes. These waste products could be degraded and recycled to recover protein, which can save our environment. One of the potential strategy to achieve this target is use of microbial biotreatment which is more convenient, cost-effective, and environment-friendly by formulating hydrolysate complexes that could be administered as protein supplements, bioactive peptides, or animal feed ingredients. Keratin degradation shows great promise for long-term protein and amino acid recycling. According to the MEROPS database, known keratinolytic enzymes currently belong to at least 14 different protease families, including S1, S8, S9, S10, S16, M3, M4, M14, M16, M28, M32, M36, M38, and M55. In addition to exogenous attack (proteases from families S9, S10, M14, M28, M38, and M55), the various keratinolytic enzymes also function via endo-attack (proteases from families S1, S8, S16, M4, M16, and M36). Biotechnological methods have shown great promise for enhancing keratinase expression in different strains of microbes and different protein engineering techniques in genetically modified microbes such as bacteria and some fungi to enhance keratinase production and activity. Some microbes produce specific keratinolytic enzymes that can effectively degrade keratin substrates. Keratinases have been successfully used in the leather, textile, and pharmaceutical industries. However, the production and efficiency of existing enzymes need to be optimized before they can be used more widely in other processes, such as the cost-effective pretreatment of chicken waste. These can be improved more effectively by using various biotechnological applications which could serve as the best and novel approach for recycling and degrading biomass. This paper provides practical insights about molecular strategies to enhance keratinase expression to effectively utilize various poultry wastes like feathers and feed ingredients like soybean pulp. Furthermore, it describes the future implications of engineered keratinases for environment friendly utilization of wastes and crop byproducts for their better use in the poultry feed industry.

Key words: keratinase, engineered keratinase, poultry feather, nutrient digestibility

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INTRODUCTION

Keratins are fibrous proteins that are the component of skeleton and epidermal tissues, having a high degree of disulfide cross-linking hydrophobic interactions, and hydrogen bonding, that are weakly susceptive and insoluble to digestion through enzymes like papain, pepsin, and trypsin (Grazziotin et al., 2006). For hydrolyzing wool, feather, and hair-like rigid protein that is keratin, it needs a special type of enzyme called keratinase enzyme (de Menezes et al., 2021). Every year, there is a large quantity of byproducts of agroindustry produced but hardly disposed of because of the highly compact structure and high contents of disulfide-bond (Su et al., 2020). Such accumulations are seen as wasting resources and raising environmental concerns (Brandelli 2008). The problem of disposing of industrial keratin waste can be addressed by the effective utilization of keratinase enzymes. Owing to potential of keratinases, various investigations has been conducted in last few decades to evaluate the effectiveness of keratinases, their production, optimum conditions and uses (Nnolim et al., 2020b; de Menezes et al., 2021; Akram et al., 2022).

By using steam pressure cooking, feather meal is prepared by some industries that need high energy consumption. In animal feed, feather meal is used at a minimum level as it lacks methionine, tryptophan and

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histidine amino acids (Luong and Payne 1977; Grazziotin et al., 2006). Despite the lowest digestibility values, variability of protein contents and bioavailability, feather meal proved as an important animal feed source (Papadopoulos et al., 1986; Laboissière et al., 2020). One option to enhance digestibility might be hydrolysis with enzymes carried out by microbes that break down keratin substrates like bird feathers. Using a feather lysate made by the bacteria (*Bacillus licheniformis)* supplemented with amino acids resulted in similar growth performance of birds comparable to soybean meal (Grazziotin et al., 2006). This enzyme significantly increased the digestibility of a commercial feather meal (Zhou et al., 2020).

Keratinases have a wide range of biochemical specificities because of the variety of resources; that reflect from the enzymatic structural makeup, max temperature pH level and stability. Commonly temperature for the reaction of keratinases is moderate as 40 to 60̊ C˙ even if the keratinases released by variants found near to hot springs and volcanoes are often thermally sensitive (Bouacem et al., 2016; Mechri et al., 2019). At the commercial scale there are so limited keratinases, that are used, many keratinases' ineffective output and low catalytic abilities make them unsuitable for use in industry (Su et al., 2020; de Menezes et al., 2021; Cai et al., 2022). These existing limitations regarding keratinase activity and availability, necessitate looking for better strategies to enhance their expression and activity to effectively degrade the different keratin- containing waste products. Production of keratinases at a commercial scale through green, feasible and economical methods will ensure the possibility of recovering dietary nutrients from wastes (feathers/wool/hair) and crop byproducts (soybean pulp and rapeseed meal etc.) with a positive impact on the global environment. There is numerous literature available on the activities of keratinases (Qiu et al., 2020a; Qiu et al., 2022) but information on production of keratinase at a commercial scale through enhancing keratinase gene expression through molecular strategies is limited. Therefore, this review aims to comprehensively summarize the strategies for enhancing the expression of keratinase, its physiological mechanism of action for keratin degradation and its potential implications for the poultry feed industry.

CLASSIFICATION OF KERATINASES

Hard tissues in both humans and animals, including feathers, yarn & wool, hair, hoofs, and nails, contain the complex and structurally stable protein that is keratin. Specially feathers and wool, are major sources of protein-rich waste that have a great potential to be turned into high-value goods like animal feed, agricultural products, or biofuels (Vidmar and Vodovnik 2018). Keratinous substrates' refractory structure and resistance to hydrolysis by conventional proteases are 2 primary obstacles preventing their valorization. However, some microbes create specific keratinolytic enzymes that can effectively destroy these forms of substrate (Qiu et al., 2020a). Keratinolytic enzymes have already been used in the leather, textile, and medicine sectors. Nevertheless, to use them more widely in other processes, such as the affordable (pre)treatment of poultry trash, the manufacturing, and efficiency of the existing enzymes must still be enhanced (Qiu et al., 2022). Here, we provide a thorough explanation of the classification, ideal circumstances needed for maximum activity, source organisms, sources, substrate number of AA Mw (kDa), etc. Different proteolytic enzymes, source organism, and substrate (kDa) (Table 1).

ESSENTIALS OF KERATIN BIOMASS AS SUBSTRATE FOR KERATINOLYTIC ENZYMES

A large amount of keratin byproducts is widely produced through slaughterhouses that may be disposed of or using expensive thermochemical methods for the creation of animal feed, poorly appreciated. Keratinolytic microbial collaborations, which take their cues from nature, offer a practical and environmentally responsible strategy to maximize this resource's endurance (Kang et

Table 1. Different proteolytic enzymes, source organism, and substrate (kDa).

Original source of keratinase gene	Host strains for cloning and Expression	Vector type	Optimum conditions	Accession number of cloned gene	Keratinase protein mol. mass	Reference
Bacillus licheniformis MKU3	*Escherichia coli BL21*	pET30b	pH 6·0 at 30C	DQ071570	3 to 47 kDa	(Kayalvizhi and Gunasekaran 2008)
Bacillus licheniformis MKU3	*Bacillus megaterium ATCC 14945*	pWH1520	pH 6·0 at 30C	DQ071570	3 to 47 kDa	(Huang et al. 2017)
Bacillus licheniformis MZK-05	*Escherichia coli BL21; Esherichia coli DH5α*	pGEX-6p-2	pH 8.5 at 55C	Q9FDF4	39 kDa	(Al Mamun 2019)
Bacillus licheniformis PWD-1	*Bacillus subtilis DB104*	pJCD	pH7.5 at 50C	S78160	33 kDa	(Wang and Shih 1999)
Bacillus circulans DZ10	*Escherichia coli BL21*	pABT2	pH 12.5 at 60-70C	KC621294	20.4-kDa	(Benkiar et al., 2013)
Bacillus pumilus KS12	*Escherichia coli*	pEZZ18	pH 7.0 at 37C	KC814159	20 kDa.	(Benkiar et al. 2013)
Geobacillus stearothermophilus AD-11	*Escherichia coliBL21(DE3)*	pTZ57R/T	pH 7.5–9.0 at 60C	KJ783444	57 kDa	(Rajput and Gupta 2013)
Pseudomonas aeruginosa	*Escherichia coli AD494(DE3) pLysS*	pET-43b(+)	pH 7.2 at 25C	-	45-53 kDa	(Gegeckas et al. 2015)
Stenotrophomonas maltophilia BBE11-1	*Escherichia coli BL21 (DE3)*	pET22b	pH 9 at 25C	KC763971	17-48 KDa	(Beyenal et al. 2003) (Fang et al. 2014)

al., 2018). Using successive experimental cultivation in keratin medium, directed selection was used to enrich soil-born microbial consortium (El-Refai et al., 2005; de Paiva et al., 2019). The enzyme could hydrolyze collagen, muscle, nails, and hair in addition to producing leucine, threonine, and tyrosine from feathers. It might be a helpful enzyme for waste treatment by encouraging the hydrolysis of the aforementioned compounds in sewage, or it might be utilized to prepare animal feed (Chitte et al., 1999; Moridshahi et al., 2021). It is produced in significant volumes as a byproduct of pig and poultry slaughterhouses. Hydrogen and disulfide bonds are used to pack keratin (Qiu et al., 2020a). Keratin can be divided into -keratin and -keratin based on the secondary structure. Keratinases (EC 3.4. peptide hydrolases) have significant potential to break down keratin for long-term protein and amino acid reuse and recycling. According to the MEROPS database, the known keratinolytic enzymes generally come from a minimum of 14 various protease families, including S1, S8, S9, S10, S16, M3, M4, M14, M16, M28, M32, M36, M38, and M55. In addition to exo-attack (proteases from families S9, S10, M14, M28, M38, and M55), several keratinolytic enzymes operate via endo-attack (proteases from families S1, S8, S16, M4, M16, and M36) (Qiu et al., 2022) or by acting solely on oligopeptides (M3, M32 family proteases, respectively) Table 2. Disulfide reductases in particular, which accelerate the breaking of disulfide bonds to improve keratinase catalytic activity, also play an essential part in keratin breakdown (Wu et al., 2022). There are a few examples of proteolytic enzymes, yet it is important to present the classification of various proteolytic enzymes thoroughly as well as any accessible information regarding substrate and enzyme action.

MECHANISM OF KERATIN DEGRADATION BY ENZYMES

Many industries have recognized biocatalysts as promising alternatives to chemical reactions. Nevertheless, the specificity, stability catalytic effectiveness, and of the enzymes' unfavorable characteristics have mostly prevented their use in industry (González et al., 2020). Disulfide bonds are used to cross-link protein chains, giving them exceptional structural strength and resistance to proteolytic destruction of keratins. Keratin breakdown in keratinaceous biomass, which are the waste products of the livestock and chicken industries, is influenced by a decrease in disulfide bonds (Shen et al., 2022; Gahatraj et al., 2023). As the primary constituent of feathers, keratin is thought to be difficult for ordinary proteases to break down, resulting in significant wastage (Wang et al., 2023b). The single enzyme with the greatest ability to degrade feathers is keratinase. Nevertheless, there is so much research work being carried out, keratinase has some limitations to use at such a large scale (Pei et al., 2023; Wang et al., 2023b). It's so much more difficult to choose an appropriate keratinase that has maximum activity and fewer limitations to overcome the industrial & environmental needs, so there is a need to exploit immediately a type of keratinase that has low pH, heat resistance, maximum activity and lower cost. Significant worth of products of degradation that are useful in the fields of the aquaculture industry, manufacturing, and pharmaceuticals. The classification, connection between developmental evolution and break down methods and modes, as well as contemporary research and application of keratinase, were reviewed. The current state of keratinases research needs further investigations to provide practical insights into their effective use. For instance, in a decreasing ecology, the majority of the chosen keratinases are unable to breakdown keratin alone without rupturing the keratin disulfide link (Nnolim et al., 2020b; Weihang et al., 2023). The mechanism underlying the complicated process of keratin breakdown is still not fully understood. The majority of research, however, indicates that the procedure of breakdown involves the breaking of disulfide bonds and the hydrolysis of disrupted proteins. Hydrophobic and electrostatic interactions cause keratinase to attach to the surface of the keratin molecule, which then initiates the catalytic process. Reductive cleavage of keratin's disulfide links, which loosens the protein's structure, increases its solubility, and exposes more protease attack sites, is the first stage of keratin breakdown. The hydrolysis of disrupted keratin is the second stage of keratin breakdown. Keratinases can break down peptide chains, but they are unable to break down individual disulfide bonds. The 14 proteases described above that are involved in the breakdown of keratin can be further broken down into endoprotease (S1, S8, S16, M4, M16, M36), exoprotease (S9, S10), oligopeptidase (M3, M32), and others (Qiu et al., 2020). These three keratinase families are necessary for the full decomposition of keratin into distinct amino acids. Oligopeptidase targets peptide bonds in short peptides, converting them into dipeptides, tripeptides, or even free amino acids. Endoproteases catalyze the cleavage of peptide bonds within the peptide. Exopeptidases attack the terminal peptide bonds (Wang et al., 2023b).

Poultry waste like feathers contributes 5 to 7% of the total mass of the chicken and has become a massive part of pollution because of its intractable nature (Tamreihao et al., 2019). The poultry and meat industries produce massive waste products like wool, feathers, hoofs, and many more waste products that would be a big source of protein, peptides, minerals, and amino acids that can be retained through different fermentation mechanisms (Gupta et al., 2023). Traditional methods or mechanisms needed a lot of energy and were expensive as compared to microbial degradation, which is low-cost and eco-friendly (Li, 2022). The putative mechanism of degradation of feathers or poultry waste through keratinolytic and proteolytic microbes is presented in (Wang et al., 2023) (Fig. 1). The keratinlytic and proteolytic enzymes that are produced by the microbes mediate the sulfitolysis for the breakdown of the disulfide bond (Qiu et al., 2020b). The sulfitolysis is carried out by the attack of disulfide reductases through cleaving down the beta-sheet disulfide bond bridge (Vidmar and Vodovnik 2018).When the 2

Table 2. The sequenced keratinolytic enzymes' protease family, accession number, source organism, degrading substrate, and a few physical traits.

NCBI accession number	Original source	Substrate	Source organism	Max Efficiency rate U/ml or μg/mL	Mw (KDa)	Optimum condition (pH, temp.)	No. of A.A	References
S1 family								
AAO06113	Tile joint	Keratin	*Nocardiopsis sp. TOA-1*	1500 U/mL	-	12.5, 60°C	384	(Nnolim et al., 2020b)
CAH05008	–	Keratin azure	*Streptomyces fradiae*	-	26	9.0, 55°C	307	(Liu et al., 2022)
S8 family								
1205229A	Soil	Keratin	*Parengyodontium album*	500−0.01 g/L	28.9	7.5, 37°C	277	(Ren et al., 2020)
AGK12420	Poultry farm	Feather	*Stenotrophomonas maltophilia*	1100 mg/L	48	8.0, 60°C	634	
AGK29593	Poultry farm	Feather	*Stenotrophomonas maltophilia*	-	40	8.0, 60°C	580	(Nnolim and Nwodo 2021)
AAK61552	Hot spring	Feather	*Fervidobacterium pennivoran*	-	130	10.0, 80°C	699	(Solanki et al., 2021)
ADD51544	Campus soil	Feather	*Thermoactinomyces sp. CDF*	400 U/mL	30	11.0, 80°C	384	(De Oliveira Martinez et al., 2020)
AAS86761	–	Feather	*Bacillus licheniformis*	-	–	–	379	(Alvarez-Vera et al., 2022)
AKR05134	–	Feather	*Bacillus amyloliquefaciens*	258.57 U /L	27	11.0, 50°C	382	(Schwabe et al., 2021)
ANQ68333	Compost	Feather	*Bacillus pumilus*	3.2 mg/L	38	–	383	(Mamangkey et al., 2020)
ANQ68334	Compost	Feather	*Bacillus pumilus*	3.2 mg/L	38	–	383	(Qiu et al. 2020)
ACC94305	Soil	Feather	*Bacillus cereus*	0.169 U/mL	80	8.5, 50°C	917	(Cui et al., 2023)
ADD64465	Sugarcane molasses	Feather	*Bacillus halodurans JB99*	3.8 mg/mL	29	11.0,70°C	361	(de Paiva et al., 2019)
ARH33809	Hot spring	Feather	*Meiothermus taiwanensis*	145.57 mg/L	41.3	10.0, 65°C	400	(Chen et al., 2020)
BAQ36632	–	Keratin azure	*Stenotrophomonas maltophilia*	500 mg·L -1	42	10.5, 50°C	589	(Cavello et al., 2021)
APY18977	Poultry compost	Feather	*Thermoactinomyces sp. YT06*	1325 U/mg	35	9.0, 65°C	389	
AAS45672	Humans	Keratin azure	*Trichophyton benhamiae*	0.049 μg/mL	39.7	–	399	(Wang et al., 2017)
AAS45666	Humans	Keratin azure	*Trichophyton benhamiae*	0.049 μg/mL	28.2	–	400	(Latka et al., 2020)
AJD23187	Horn	Pig bristle	*Onygena corvina*	6.65 U/mL	–	–	393	(Pilgaard et al., 2019)
AJD23193	Horn	Pig bristle	*Onygena corvina*	-	–	–	369	(Huang et al., 2020)
CAD24008	–	Cat keratin	*Microsporum canis*	-	51.3	–	485	(Khedmati et al., 2023)
CAD24009	–	Cat keratin	*Microsporum canis*	0.5 g \ L	46.1	–	427	(Qiu et al., 2020)
CAD24010	–	Keratin azure	*Microsporum canis*	-	31.5	–	397	(Akram et al., 2022)
S9 family								
S10 family								
AAS76667	Clinical isolate	Keratin	*Trichophyton rubrum*	-	90	–	652	(Khedmati et al., 2023)
AAS76666	Clinical isolate	Keratin	*Trichophyton rubrum*	-	85	–	662	(Datt et al., 2021)
S16 family								
AMW33508	Hot spring	Feather	*Fervidobacterium islandicum*	4.4 g/L	88	–	631	(Kang et al., 2020)
M3 family								
AJD23200	Horn	Pig bristle	*Onygena corvina*	-	–	–	783	(Huang et al. 2020)
M4 family								
ADP00718	Garden soil	Feather	*Pseudomonas aeruginosa*	20 mg/L	–	–	475	(Ramalingum et al. 2022)
AJD77429	-	Keratin azure	*Geobacillus stearothermophilus*	-	57	9.0, 60°C	546	(Peng et al. 2023)
M14 family								
ABG67896	Clinical isolate	Keratin	*Trichophyton rubrum*	-	42	–	422	(Zhang et al. 2020)
M16 family								
AMW32060	Hot spring	Feather	*Fervidobacterium islandicum*	4.4 g/L	44	–	406	(Kang et al. 2020)
M28 family								
AAS76670	Clinical isolate	Keratin	*Trichophyton rubrum*	-	58	7.0, 50°C	373	(Huang et al. 2018)
AJD23165	Horn	Pig bristle	*Onygena corvina*	6.65 U/mL	-	-	374	(Huang et al. 2018)
CAH03796	–	Feather	*Streptomyces fradiae*	17,399 U/mL	36	8.0, 60°C	461	(Lu et al. 2020)
AJD23207	Horn	Pig bristle	*Onygena corvina*	-	-	-	493	(Huang et al. 2018)
AAS76669	Clinical isolate	Keratin	*Trichophyton rubrum*	-	33	7.0, 50°C	495	(Huang et al., 2018)
M36 family								
BAM84176	Soil	Wool cuticle	*Fusarium oxysporum*	1 g.L-1	46.8	7.0, 50°C	632	(Dhanasingh and Lee 2019;
CAD35288	–	Keratin azure	*Microsporum canis*	0.5 g \ L	-	-	633	(Qiu et al. 2020)
AJD23141	Horn	Pig bristle	*Onygena corvina*	6.65 U/mL	-	-	634	(Huang et al., 2018)

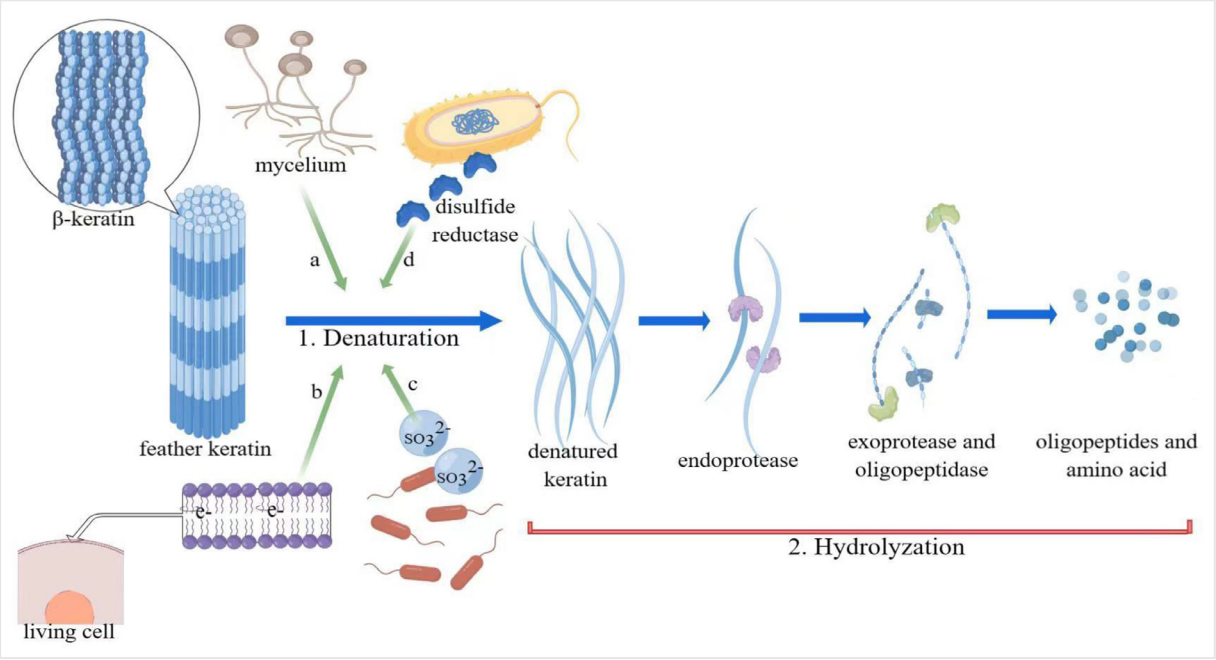

Figure. 1. Its shows the degradation mechanism of feather keratin. The feather keratin is a type of β-keratin and has a lot of disulfide bonds. There are 2 steps in the process of keratin breaking down: denaturation and hydrolyzation (Wang et al., 2023).

strains of keratinolytic and disulfitolytic enzymes produced by microbes (bacterial or fungal) perform the activity cooperatively, the degradation rate is increased gradually, but maintenance of pH is most important in this aspect of the mechanism of degradation of waste keratin source, whether the keratinolytic and disulfolytic strains of enzyme degradation are thermostable (Brandelli et al., 2010). The characteristics of microbial strains, keratinase nature, optimum temperature and maximum pH for degradation activity is presented in Table 3.

POTENTIAL APPLICATIONS OF KERATINOLYTIC ENZYMES IN POULTRY FEED INDUSTRY

Yearly there are tons of wastes generated by the poultry industry containing keratin degraded leading to serious environmental hazards. Keratins are comprised of α- and β- types which give support and durability to their structural complexities and prove themselves proteins with fibers that are extremely malleable (Akram et al., 2022; Liya et al., 2023). The discovery of keratinase, a wide-ranging protease from thermally sensitive fungi and bacteria, has made it possible to convert the peptide bonds in extremely resistant keratinous materials like nails, feathers, claws, and horns into beneficial amino acids in an environmentally acceptable manner. These enzymes that break down proteins are produced by microbes using solid-state fermentation & submerged processes (Akram et al., 2022). Protein-breaking enzymes i.e. keratinases, have special characteristics to break down the peptide bond of keratin protein containing bio-waste and convert it into another type of protein, all these processes may be possible through the vast range of activity that effectively converts keratin into valuable

Table 3. Characteristics of keratinase produce by keratinolytic bacteria and fungi sp.

Microbe strain	Keratinase nature	Max. temp. (°C)	Optimum pH	References
Bacterial strains				
Fervidobacterium islandicum	Serine	70-90	9.0	(Dhanasingh and Lee 2019)
Chryseobacterium sp.	Metallo	50	8.5	(Deniz et al., 2019) (Alwakeel et al., 2021)
Bacillus pumilus	Serine	60	10	(Tork et al., 2010) (Dhiva et al., 2020)
Bacillus pseudofirmus	Serine	60	8.8−10.3	(Kojima et al., 2006) (Chen et al., 2020)
Bacillus sp	Serine	30-37	7-8	(Lee et al., 2002)
Bacillus licheniformis	Serine	50	7.5	(Nnolim and Nwodo 2021b)
Bacillus pumilis	Serine	60	10.5	(Jayalakshmi et al., 2010)
Actinomyces sp	-	28	7.5	(Jagtap et al., 2020)
Fungal strains				
Aspergillus stelliformis	Serine	50	8	(Timorshina et al., 2022)
A. stelliformis	Serine	40		
F. brachygibbosum	Serine	30	6	
A. flavus	Serine	28	8	(Alwakeel et al., 2021)

constituents. Keratinous substances like horns, hair, feathers, etc. have received particular focus among these protein residues, it is not thought to be environmentally friendly when disposed of using harmful procedures like acid/alkaline hydrolysis or incineration (Ossai et al., 2022; Peydayesh et al., 2022). Under moderate circumstances, keratin can be broken down by microbial keratinolytic enzymes, producing keratin hydrolyzed products that nevertheless contain intact amino acids & peptides, the quest for fungal & bacterial genera that can effectively manufacture keratinases might help to ensure the sustainability of business because of the relevance of these enzymes to science and their potential application in green technologies (de Menezes et al., 2021).

IMPROVEMENT OF NUTRIENT DIGESTION BY USING KERATINASES

Due to the creation of enzymes that are specifically created to function efficiently in the bird's stomach and are feed mechanism stable, the utilization of innovation in this field has advanced over the last 10 to 12 yr. A while ago, when added to diets rich in wheat and soybean meal, enzymes such as endo-mannanases and endoxylanases also greatly enhanced the growth efficiency of chicken (Jagadeesan et al., 2020). Dietary supplements generally increase body weight growth and feed conversion rates. When keratinase fortification was given to broiler chicks fed diets with low or appropriate protein levels, their growth efficiency improved. It is necessary to conduct more research on ideal digestibility and utilization of nutrient capability to better understand the advantages of keratinase administration (Rao et al., 2020). Additionally, keratinase administration enhanced food digestibility, intestinal shape, and intestinal ecology while decreasing the frequency of diarrhea and the release of pro-inflammatory cytokines (Wang et al. 2011). The addition of protease irrespective of sorghum or corn-based diets, increased protein digestibly and potentially sustained gut health condition confirmed by probable decreased oxidative stress and enhanced morphology (Chen et al., 2017).

EFFECT OF KERATINASES ON POULTRY FEATHERS AND PROTEIN INDREDEINTS

Agriculture's sustainable development benefits both the environment and the economy. In modern agriculture, boosting the efficiency of nutrient utilization is also important because nitrogen is the essential nutrient responsible for the most environmental stress (Ladha et al., 2020). To prevent losses of naturally produced components to feces and provide highly digested protein feed with a high degree of bioavailability. Local sources of protein can help to cut down on transportation-related greenhouse gas emissions (Song et al., 2018). By producing highly digestible feed, excessive nitrogen fluxes because of environmental contamination are reduced. Feed protein reduction and amino acid supplementation are 2 other strategies for reducing ammonia emissions from animal farming. Innovative animal feeding must be used to lessen agricultural harmful environmental effects. These developments are associated with both the product (a particular kind of feed) and the strategy for livestock feeding (Muller et al., 2017). It is being tried to substitute exported soybeans with high-protein feeds to cut down on transportation-related greenhouse gas emissions. Additionally, it permits the use of nearby resources for protein feeding. It's essential in a crop-livestock integrated system because it reduces nitrogen discharge. After all, nutrient cycling is limited to a narrow region (Yu et al., 2019). The replacement of traditional protein diets with ones that have a lower crude protein concentration and are supplemented with the required amino acids is one possible approach. All of these initiatives attempt to lessen greenhouse gas emissions and ammonia, which will lessen the ecological impact of agriculture (Chojnacka et al., 2021).

EFFECT OF KERATINASE SUPPLEMENTATION ON GROWTH PERFORMANCE OF BROILERS

Keratinase supplementation can improve the growth performance of birds fed low-protein diets by improving the nutrient digestibility values (Ladha et al., 2020). The PWD-1 keratinase broad-spectrum enzyme has shown a significant growth effect in broilers at the starter phase on corn-soyabean-based diets (Odetallah et al., 2003). The 3 different regimes of treated and untreated feather diet in broiler chickens proved significantly good results in growth performance, chicken health, carcass properties and meat quality. So, feather meal for poultry chickens may be used as an effective diet regime (Ayanwale et al., 2023). The feather-based diet in broiler chickens investigated under the 2 different regimes keratinase supplement diet visibly improved the Glutathione activity and retard the Melanodialdehyde amount in wing muscles, and found that the highest regime proved good in meat quality, digestibility, and growth of feather-containing diet (Xu et al., 2023). Protease diet in broiler chickens are studied is significantly better results in diet conversion ratios, diet intake body weight internally was not affected but it proved the better- improved result for the morphology of guts (Lee et al., 2022). Specific bacteria, such as *Firmicutes, Bacteroidetes, Porphyromonadaceae, Lachnospiraceae, Bacteroidaceae,* and *Ruminococcaceae*, experienced a shift in density as a result of protease addition. Its suggested that the protease supplementations at 50g/ton, have improved the performance of gut and gut morphology through changing the abundance of the useful bacteria strains (Huyan et al., 2022). Effect of various proteases (Keratinase) enzymes on the performance of poultry birds Table 4.

Table 4. Effect of various proteases (Keratinase) enzymes on the performance of poultry birds.

Enzyme source	Feed	Type of bird	Duration of experiment	Effect of enzyme	References
Aspergillus niger CCUG 33991	Corn- or a wheat-based diet	Ross 308 male broiler	42 d	• Increased the body weight gain. • Improved the feed intake. • Enhanced the FCR. • Improved the ileal digestibility of dry matter. • Enhanced the crude protein. • Increased the gizzard and villus.	(Ghayour-Najafabadi et al., 2018)
Aspergillus oryzae	Feather meal & soy meal	Broiler chicken	28 d	• Having good potential for weight gain. • Enhanced the crude protein. • Increase gut & gizzard performance.	(Brandelli et al., 2015) (Golunski et al., 2016)
Aspergillus Oryzae GB-107	Corn, rice brain & Soybean meal	Broiler chicken	30 d	• Improved gut health. • Improved feeding. • Enhance weight gain. • Enhanced the crude protein.	(Soomro et al., 2017) (Li et al., 2020)
Rhizopus, & *Penicilium*	Wheat bran, rice husk, sour cherry kernel	Broiler chicken & Layer	21 d	• Improved immunity. • Improved weight gain. • Better feeding intake. • Improved digestibility. • Increase gut & gizzard performance.	(Sun et al., 2022) (Zhu et al., 2023)
Bacillus licheniformis LMUB05	Feather meal	Broiler chickens	21 d	• Depress the body weight in control. • Improved feeding. • Significantly better digestibility. • Improve gut function.	(Adetunji et al., 2018) (Sypka et al., 2021)
Bacillus licheniformis PWD-1	Corn & soybean meal	Broiler Male & female chicks	21 d	• Decrease in jejunum viscosity, • Increase FCR. • Improved food conversion. • Weight gain improve.	(Odetallah et al., 2003b)
Bacillus subtilis	Corn & soybean meal	Arbor Acres Male broiler	31-d	• Increased weight gain. • Improved feed intake. • Improved IVCPD. • Increase FCR.	(Zheng et al., 2023) (Huyan et al., 2022)

Fungal and bacterial fermented proteases play a tremendous role in poultry feed formulation that proven relatively strong effects on immunity, weight gain, gut and gizzard performance, and feed conversion rate in broiler chickens (Ghayour-Najafabadi et al., 2018). Corn- or wheat-based diet supplementation with bacterial xylanase or fungal xylanase has no effect that is worth mentioning on performance or productivity in the starter period of broiler chickens (Fries-Craft et al., 2023). Beta-mannanase has significant effects on health, performance, pododermatitis, intestinal morphology, and digestibility through *Clostridium perfringens*. A wheat and soybean-based feed supplemented with xylanase and beta-mannanase administration in Ross 308 chickens proved good in weight gain and gut health (Abd El-Wahab et al., 2022). The potential benefits regarding the efficiency and performance of poultry compared with traditional and keratinase supplemented diets is presented in Figure 2.

The effects of 2 different types of phytase enzymes in corn and wheat-based diets on growth, microbial community in the cecum, characteristics of carcass and bones have been investigated. Chickens cannot manufacture phosphorus from plant phytate; the phytase enzyme must be added to their meals. *Saccharomyces boulardii*, a recombinant probiotic that produces recombinant phytase locally, as a dietary supplement (Salmanian et al., 2022). Supplementation with protease proved effective in the growth, performance, gut morphology and organ development of male broiler chickens. It also enhanced the body weight, gut activity and immunity responses (Huyan et al., 2022). Protease supplementation improved daily feed intake and gut profiles and changed the relative abundance of bacterial traits in *Ruminococcaceae, Porphyromonadaceae, Bacteroidaceae*, and *Firmicutes* (Oyeagu et al., 2023).

METHODS TO ENHANCE KERATINASE EXPRESSION

The primary component of poultry feathers is keratin, a protein rich in β-pleated sheets that make it resistive to the proteolysis process. Despite the identification of numerous keratinases, the reasons behind their substrate specificity towards β-keratin are still unknown because it is challenging to prepare a soluble feather keratin substrate for application in

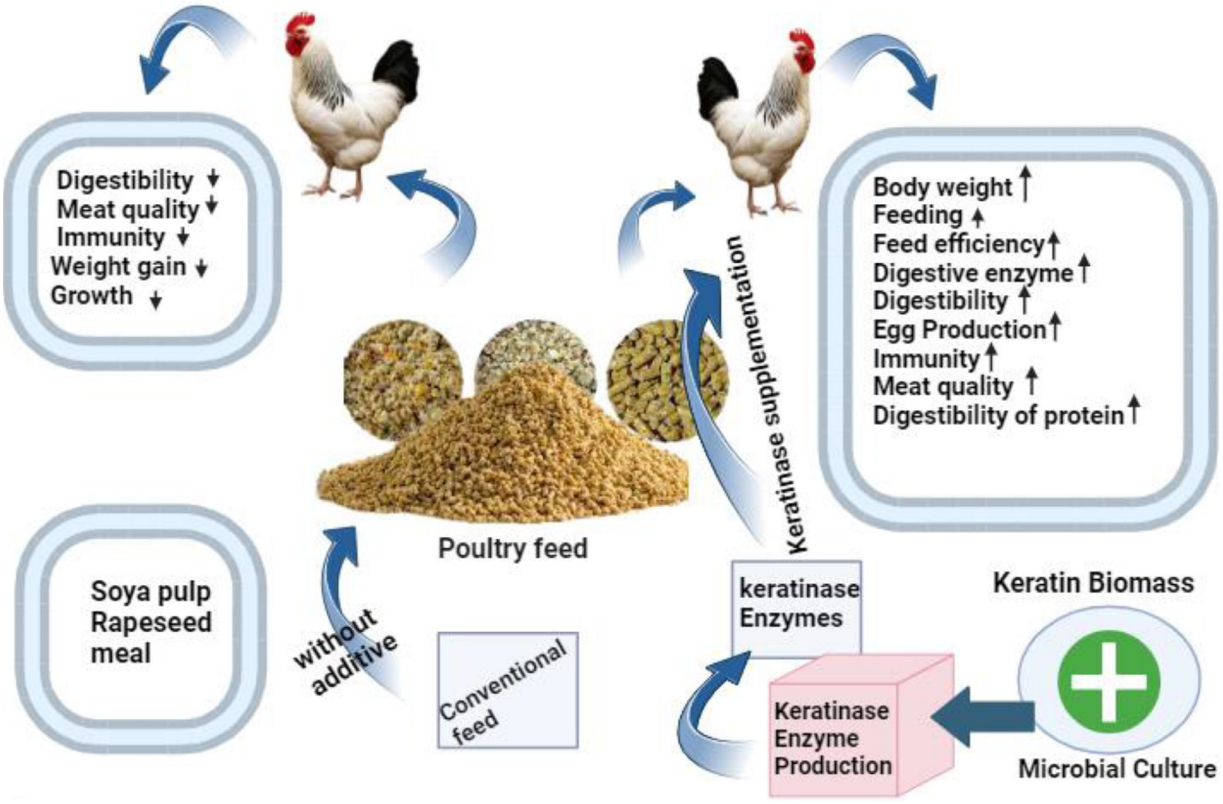

Figure. 2. The efficiency and performance in poultry compared with traditional and keratinase supplemented diet.

activity tests (Jin et al., 2017; Parinayawanich et al., 2021). Using purified recombinant keratin and casein as substrates, the proteolytic activity of crude extracts from the feather-degrading bacterium *Fervidobacterium islandicum* AW-1 with proteinase K, trypsin, and papain. While all proteases that were studied showed significant proteolytic activity for casein, only proteinase K and crude extracts from F. islandicum AW-1 demonstrated significant keratinolytic ability for the recombinant keratin (Sharma and Kango 2021). Different microbial strains are used in expressing the keratinase enzymes, including *Bacillus licheniformis* MKU 3, *Bacillus licheniformis* PWD-1, *Bacillus licheniformis* MKU 2, *Bacillus subtilis* BF20, *Bacillus mojavensis, Bacillus tequilensis* Q7 and many more that are used in modern biotechnological preparation for keratinase expression (Yong et al., 2020). Due to the increased demand for protein and protein products, the importance of different methods to sort out new sources that are potentially and economically flexible for protein availability, so we are discussing some important strains that are potentially important for the expression of keratinases in different methodologies involving maximizing keratinase gene expression.

USE OF GENETICALLY MODIFIED MICROBIAL STRAIN

Genetic engineering can be used to enhance the keratinase production. Gene deletion and random or site-specific mutagenesis could be used to modify several natural host system components to boost keratinase productivity. Random mutagenesis of *Brevibacillus sp.* strain ASS10II by UV light treatment led to the increased keratinase synthesis (Devi et al., 2023). Furthermore, ethyl methanesulfonate-induced random mutagenesis of Candida parapsilosis resulted in a mutant strain that was superior to the original strain in terms of keratinolytic activities(Sharma et al., 2023). Furthermore, it has been demonstrated that regulators of transcription are essential for controlling the expression of heterologous genes (Sharma et al., 2023). In B. subtilis, it has been discovered that mutating transcription factors or small RNA genes (**sRNA**) like ccpA and codY enhances the expression of heterologous protein. Furthermore, significant production of aprE was generated by the deletion of antisense sRNA aprA, which controls aprE gene expression of the extracellular protease (Stühmeier-Niehe et al., 2023). In the altered strain, the removal of Bli03719 RNase enhanced the expression of heterologous genes. Therefore, the elevated expression of recombinant keratinases from a native host might be achieved using the same methodology (Friedrich et al. 2023).

The xylose induction of expression approach in *B. megaterium* with the T7 promoter in *E. coli* allowed for the effective cloning and expression of *Bacillus licheniformis* keratinase (Rahimnahal et al., 2023). Following response surface technique optimization of the process settings, 3 times as much keratinase was generated by the recombinant *B. megaterium* (pWHK3) as by *B. licheniformis* MKU3 (Radha and Gunasekaran 2009). The keratinase recombinant strain can be produced

with *B. megaterium*, which is also a suitable host for the effective expression of genes that can be improved through protease preparations (Radha and Gunasekaran 2007; Kieliszek et al., 2021). The selection of the proper host for the preparation of recombinant enzyme protein, which is expressed in bacterial strains such as lactic acid bacteria, *Bacillus*, and *Escherichia coli* and used in *Pichia pastoria* (yeast) and *Aspergillus* (fungi), is the primary factor that can affect the yield and production rate (Liu et al., 2013; Hou et al., 2019). Recombinant enzyme proteins are easier to express in bacterial hosts since they can't be overexpressed and require modification after translation (Gong et al., 2020; Ruiz-Villafán et al., 2023).

With the use of the T7 promoter in *E. coli* and the xylose induction of expression method in *B. megaterium, Bacillus licheniformis* keratinase was successfully cloned and expressed (Liu et al., 2013). The process parameters were optimized using response surface methods, and the recombinant B. megaterium (pWHK3) produced 3 times as much keratinase as *B. licheniformis* MKU3 (Radha and Gunasekaran 2009). The *B. megaterium* is an appropriate host for the successful expression of genes which can be optimized through protease preparations by the use of *B. megaterium* that is further suitable to produce keratinase recombinant strain (Radha and Gunasekaran 2007; Kieliszek et al., 2021). The main factors that may affect the yield and production rate depend upon the choice of appropriate host for preparing recombinant enzyme protein that is expressed in bacteria strains like lactic acid bacteria, *Bacillus* and *Escherichia coli,* it would be used in *Pichia pastoria* (yeast) and *Aspergillus* (fungi) (Liu et al., 2013; Hou et al., 2019).In a bacterial host, it's more convenient to express recombinant enzyme protein as it cannot over express large proteins that may need to post translational modifications (Gong et al., 2020; Ruiz-Villafán et al., 2023).Through xylose inducible expression arrangements in *B. megaterium* and *E.coli*; keratinase of *Bacillus licheniformis* was cloned successfully with the use of 17 promotors (Radha and Gunasekaran 2007; Gong et al., 2020). Response surface methodology (**RSM**) had been used to maximize the process that inference in 3-time high keratinase production through B. megaterium (pWHK3 recombinant than that of strain *B. licheniformis* MKU3 (Radha and Gunasekaran 2007; Tian et al., 2019; Su et al., 2023). Many studies have reported the different factors affecting the keratinase enzyme activity of different strains of bacteria on various waste products of poultry industry. It was revealed that enzyme activity was maximum at 37 to 40° C and in the pH range from 7.5 to 8.0, eventually indicating that temperature and pH significantly affects the enzyme activity and activity (Tork et al., 2010; Naseer et al., 2022). Enzyme mediated processes are pave way to improve sustainable food resources. It uses and recycles our different waste products for getting rid of anoxationary agents of pollution, so, they work in environmentally convenient way. The different additives to enzyme structures proved fruitful to make them thermostable, tolerant and resistant to denaturation at different stressful conditions (Sharma et al., 2019).

Stable strains of *Bacillus licheniformis* with several copies of the keratinase gene in their chromosomes were created to boost keratinase output. The integrated vectors containing kerA, either with or without the P43-promoter, were created and subsequently subcloned into *Bacillus subtilis* DB104 and *B. licheniformis* T399D. Southern blot analysis was used to identify and determine the presence of multiple copies of kerA integration into the chromosome in T399D (Rahimnahal et al., 2023; Su et al., 2023). Three to 5 copies were found to be the ideal range for kerA integration. Reduced extracellular keratinase processing and secretion was the result of higher integration of gene copies (>5). Instead of being incorporated into the chromosome, kerA in DB104 was cloned within the plasmid. In addition to boosting keratinase output in plasmid-based expression in DB104, the potent constitutive promoter P43 significantly enhanced the enzyme yield of the T399D integrants (Lai et al., 2023). Higher amounts of medium substrate were shown to improve cell growth and enzyme output in new strains. Upon cultivation on either soy or feather medium, the keratinase activity remained consistent and increased by approximately 4 to 6 times (Wang et al., 2004).

Keratinase can break down resistant keratinous wastes to create useful, recyclable keratin hydrolysates complexes. In addition to its industrial importance, keratinase is a valuable substitute for reducing contamination of the environment resulting from chemical treatments of keratinous wastes (Riffel et al., 2003; Brandelli et al., 2010). The current limited bioproduction of keratinase from native keratinolytic hosts hinders the enzyme's large-scale application (Chen et al., 2021). To increase the amount of keratinase production, simple methods such as cloning and expressing recombinant keratinases from natural keratinolytic hosts are used. This, however, still falls short of making up for the fact that it isn't produced on a large scale to mitigate the industrial needs (Gupta et al., 2002). Thus, the purpose was to illustrate the many sources of keratinase as well as methods for boosting keratinase synthesis in native keratinolytic hosts. Additionally, chromosomal integration, promoter engineering, plasmid selection, signal peptide and propeptide engineering, codon optimization, glycoengineering (Chen et al., 2022), and other molecular techniques to boost recombinant keratinase production were elucidated because *Escherichia coli, Bacillus sp.,* and *Pichia pastoris* are the most commonly used heterologous propagation hosts for keratinases. These strategies have been applied to these hosts to further intensify the production of recombinant keratinases that are better suited to the large-scale demand for them (Li, 2022; Yahaya et al., 2021a).

GENETIC MANIPULATION

Keratinases from a variety of native hosts, including *Fervidobacterium spp., A. viridilutea, P. aeruginosa,*

Streptomycin species and Bacillus species have been cloned and expressed and Cloning and expression of *Thermophilus* sp. in heterologous host, including *P.pastoris* and *E.Coli* have been done to enhance the recombinant keratinase output or to undergo further augmentation (Friedrich et al., 2023) Many molecular techniques have been used, mostly in common heterologous hosts like *E. Coli, B. subtilis,* and *P. pastoris*, to completely realize the use of keratinase for commercial applications (Gahatraj et al., 2023). It is possible to increase the synthesis of keratinase by genetic engineering. Several natural host system components could be altered by gene deletion and random or site-specific mutagenesis to increase keratinase production. Increased keratinase synthesis resulted from random mutagenesis of *Brevibacillus sp.* strain ASS10II by UV light treatment (Devi et al., 2023). Candida parapsilosis that is produced by a mutant strain through ethyl methane sulfonate induced random mutagenesis showed tremendous keratinolytic activity than the original strain (Sharma et al., 2023). Transcription regulators are very essential for regulating heterologous gene expression (Sharma et al., 2023). It has been found that altering short RNA genes (sRNA) such as codY and ccpA or transcription factors increases the expression of heterologous proteins in B. subtilis. Moreover, the deletion of antisense sRNA aprA, which regulates the extracellular protease's aprE gene expression, resulted in a notable increase in aprE output (Stühmeier-Niehe et al., 2023).

Heterologous gene expression was improved in the modified strain by the elimination of Bli03719 RNase. Consequently, employing the same approach may result in increased expression of recombinant keratinases from a native host (Friedrich et al., 2023). Several natural host system components could be altered by gene deletion and random or site-specific mutagenesis to increase keratinase production. Increased keratinase synthesis resulted from random mutagenesis of *Brevibacillus sp.* strain ASS10II by UV light treatment (Devi et al., 2023). Random mutagenesis of Candida parapsilosis is induced by ethyl methanesulfonate which would be proven better for keratinolytic activity (Sharma et al., 2023).

The worldwide interconnections and phenotypic impacts of transcriptional factors in bacteria have been extensively researched. *Bacillus subtilis* in particular has been utilized extensively as a model Gram-positive bacterium to describe the expression of keratinase (Jagadeesan et al., 2020). Nowadays, Bacillus species are employed as effective commercial microbial platforms to generate a wide range of metabolites, including industrial compounds, surfactants, extracellular enzymes, and heterologous proteins. From a biological procedure & perspective, however, the pleiotropic effects resulting from the genetic alteration of particular genes that codify for global regulators (transcription factors) have not been widely implicated (Jagadeesan et al., 2020; Abdelmoteleb et al., 2023). These tactics have gained prominence recently in Bacillus species due to their potential to boost the production efficiency of certain metabolites that are of industrial importance. The use of genetic engineering (mutations, deletions, or overexpression) to global regulators like Spo0A, CcpA, CodY, and AbrB has been increasingly popular recently. This trend can be advantageous for the development or enhancement of biotechnology that uses Bacillus species as manufacturing platforms (Abdelmoteleb et al., 2023). To link the relationships between these regulators—which are crucial to take into account for use in the improvement of metabolites of commercial interest—genetic networks, regulation pathways, and their relevance to the development of growth stages are also reported (Xiang et al., 2020; Abdelmoteleb et al., 2023). The reported yields from these products, which are presently produced primarily in laboratories and to a lesser extent in bioreactors, are also examined to provide insightful information about their possible applications and the developmental stage aimed at enormous-scale process improvement (Tolibia et al., 2023).

PROTEIN ENGINEERING

The following 3 approaches can be used to enhance the expression of a particular protein through protein engineering.

Pro-Peptide Optimization

Proteins' pro-peptides are crucial in aiding in their development and substantial structural remodeling. An inert propeptide-enzyme complex can be formed when the propeptide is coerced into binding to the enzyme's active site. The expression of keratinase can be increased by accelerating the process of enzyme maturation by altering specific locations of the prepeptide, which in turn changes the protein folding rate. It has been reported that the aromatic hydrophobic amino acid may be replaced with Tyr at the propeptide cleavage site (P1), which resulted in an acceleration of the release of the active mature enzyme and an increase of 3-fold in activity (Su et al., 2019). Targeted mutagenesis and gene shuffling are 2 genetic engineering strategies that can be used to change the propeptides of keratinases, resulting in a higher yield and a greater number of mutants with distinct characteristics. Keratinase belongs to the protease family, which also includes metalloproteinases and serine proteinases. Additionally, proteinase itself has the potential to hydrolyze (Yahaya et al., 2021b). Proteases are typically released as proenzymes, transmembrane under the direction of signal peptides, and then folded in the periplasmic space to prevent such uncontrolled proteolysis (Rahman 2023). This mechanism involves the propeptide, which contains the inhibitory N-terminal propeptide, and is crucial in directing its proper folding (Song et al., 2023). The metalloproteinase family often has an inhibitory N-terminus, which suppresses the action of the protease and prevents it from folding into the

correct shape before the enzyme is activated (Kawase et al., 2018; Song et al., 2023). Serine proteases also exhibit this type of inhibition (Kawase et al., 2018). As a result, propeptide sequence variation has a significant impact on enzyme function, and propeptide optimization is crucial for increasing exocrine enzyme secretion and activity. Researchers examined the function of the prepeptide sequence and the impact of its deletion, starting with *Bacillus licheniformis* BBE11-1 keratase. They came to the conclusion that the prepeptide's "chaperone effect" and "cutting efficiency" together influenced the activity of the mature enzyme. By means of site-directed modification of the propeptide and multiple sequence alignment, 7 target sites were identified, which have the potential to enhance the activity by 16% to 66% mature enzyme. At last, the enzymatic efficiency reached 391.6 KU/mg (Peng et al., 2021). The function and destination of the propeptide, as well as its function as a molecular chaperone directing protein folding and its ultimate destination of excision following enzyme activation, are all vividly described by this discovery. Precursors are really sometimes referred as intramolecular chaperones (Schaller et al., 2018). Protease optimization is as important as molecular chaperone optimization. Su et al. (2019) found that keratinase activity rose from 179 U/mL to 1,114 U/mL after they modified 18 sites in the propeptide of the enzyme and created a multisite mutation library with 6 possible sites. When fermentation conditions were optimized in addition to measuring enzyme activity, the result was at least 3,000 U/mL. Performance changes will also result from structural modifications to the propeptide itself in addition to the mutation of the propeptide location. To change the propeptide's structure, a ligand, a single amino acid substitution, and a single residue deletion were added to the C-terminal. The cleavage effectiveness of the pre-peptide was discovered to be modified by optimizing the pre-peptide excision site residues, and the mutant Leu(P1)'s Ala enzyme yield was 50% greater than that of the normal variant (Liu et al., 2014). These findings imply that the main structure of the propeptide's C-terminal is also essential for the synthesis of fully developed keratinase. C-terminal modification engineering could possibly be a useful strategy for boosting keratinase yield by eliminating the body's mutation (Li et al., 2013). It is well established that to form the mature enzyme, the N-terminal propeptide of the serine alkaline protease Sfp2 must undergo self-catalytic cleavage at the C-terminal of the P1 amino acid. To raise mature Sfp2 expression levels, it substituted a single amino acid at several places. The mutant's activity was 9 times greater than the wild types, and the other experimental groups' activities were likewise 2 to 9 times higher, respectively (Li et al., 2013). Propeptides are virtually entirely responsible for the exocrine of keratins and even the entire protease family, as the aforementioned cases make clear. For this reason, optimizing propeptides will also optimize keratins. Figure 3 depicted the schematic mechanism of propeptide assisted folding pathway.

Signal Peptide Optimization

There is no question about the significance or priority of signal peptides in the process of protein secretion. Currently, the twin-arginine translocation (**TAT**) and the general secretory route (**SEC**) are recognized as the 2 primary secretion processes in bacteria (Mendel and Robinson 2007; Kang and Zhang 2020) The SEC pathway is used to secrete the majority of these proteins (Walker et al., 2015). On the other hand, signal peptides are essential players in the secretion routes of Tat and Sec. In the case of SEC, the signal peptide's positive charge attracts and interacts with the negative phospholipid membrane structure, directing the new peptide chain to finish the transmembrane transport link. Subsequently, the h domain's ring structure opens, allowing the partially folded protein to enter the membrane structure (Thomas and Tampé, 2018). Afterwards, SPACE I cuts the signal peptide during translocation in the cell membrane or shortly after translocation, and SPPase degrades it. The mature protein will fold into the proper natural shape within the periplasmic space once the translocation is finished (Ismail and Illias, 2017). The protein will finish folding in the Tat pathway first, and then transmembrane transport is completed through the transport channels (Berks et al., 2003; Frain et al., 2019). It is evident that signal peptide performance and quality have a major impact on protein secretion, and signal peptide optimization is crucial for increasing protein secretion and activity (Freudl, 2018; Low et al., 2013). Many researchers have investigated the replacing signaling peptides because most target genes in the process of heterologous protein expression are not from the same species, which means that their native signaling peptides might not be able to function as well (Desai et al., 2010; Watts et al., 2021). The best course of action in this situation is to change to a more suitable signal peptide. The conventional approach involves constructing a library of signal peptides and utilizing rapid screening to accomplish the optimal signal peptide matching task (Chen et al., 2021; Wang et al., 2021). It was discovered that the 6 candidate signal peptides that were used to substitute KerP's natural signal peptide had increased the activity of KerP by 1.55 times. Apart from substitution, the initial signal peptide might also undergo modifications, including enlarging the matching sequence or undergoing a mutation (Tjalsma et al., 2000). The n/c terminal domain of KerSMD was substituted by Fang et al. with the homologous protease KerSMF. The mutant protein's activity may increase 2-fold upon replacement of the n-terminal domain (Akram et al., 2022). For the investigation, targeted optimization of surface signal peptides is required. Finding the appropriate signal peptide for each target protein through screening and identification is the best strategy

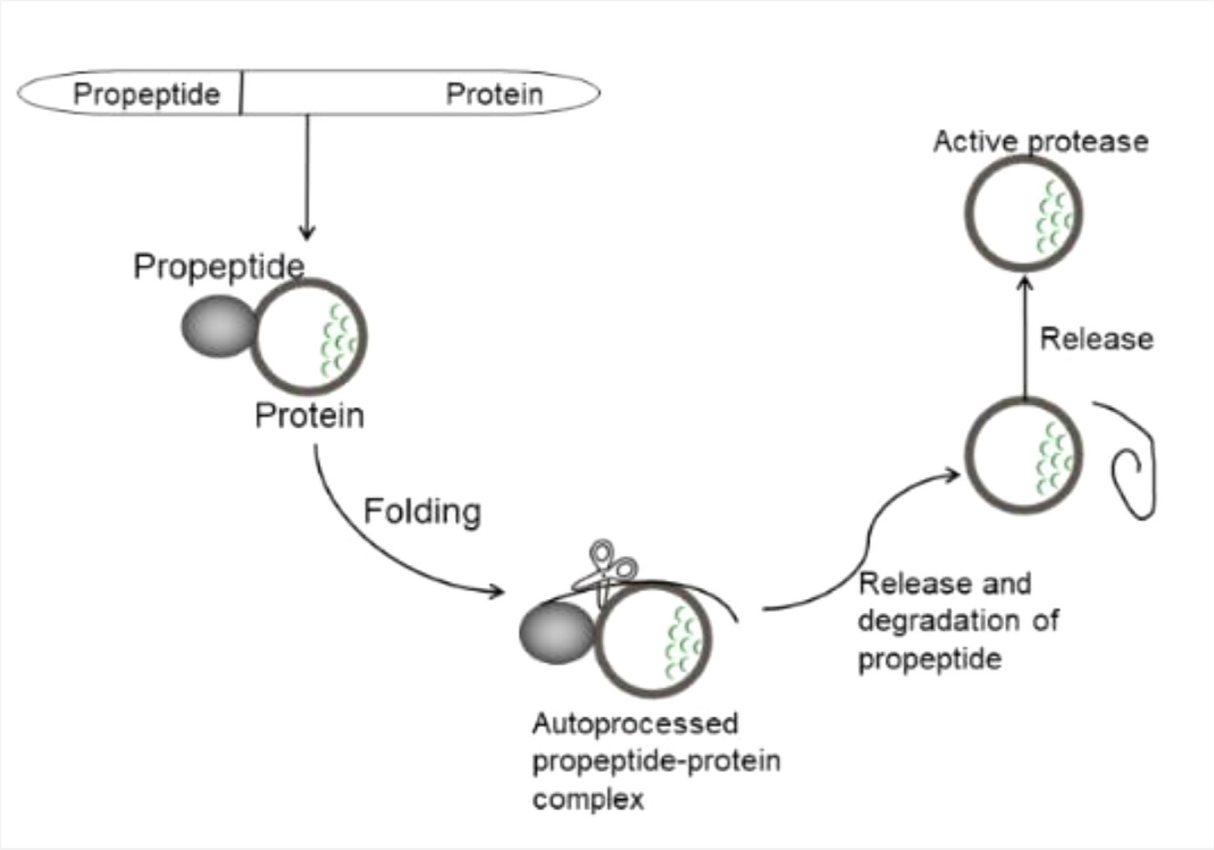

Figure. 3. Propeptide assisted folding pathway. The expression of keratinase can be increased by accelerating the process of enzyme maturation by altering specific locations of the prepeptide, which in turn changes the protein folding rate.

because different target proteins often only recognize one ideal signal peptide (Ramezani et al., 2017). Consequently, research in the optimization of keratinase secretion is concentrated on signal peptide optimization due to its significant role (Gong et al., 2020).

The host cell's vital metabolic functions and structures may be disrupted by intracellular accumulation of keratinase, which acts on metabolism-related enzymes and cell membrane proteins to cause cell lysis and death. Insufficient secretion may impede the rapid expression and production of keratinase (Brown, 2019). Consequently, effective secretion is essential for the synthesis of keratinase. Along with promoters and a few regulatory elements, signal peptides are signaling sequences that are crucial for protein secretion (Song et al., 2015). They typically consist of a dozen or so amino acids and are involved in the proper folding of the protein precursor, as well as being a crucial part of protein across the membrane transport (Greenwald and Riek 2012). The capacity of the protein to fold appropriately and secrete normally outside of the cell depends on the signal peptide's dependability (Freudl 2018). The main factor in boosting the heterologous expression of keratinases, particularly in the Bacillus expression system, is screening and optimizing the best signal peptide (Fu et al., 2018). Proteomic study of *Bacillus subtilis* revealed that the bacterium has 4 secretion routes and secretes over 300 proteins elsewhere (Tjalsma et al., 2004; Aguilar Suárez et al., 2021). Using cyclodextrin dextranase expressed in *Bacillus subtilis* as the host, optimized the signal peptide and promoter, screening and selecting YdjM and P-aprE as the best signal peptide and promoter, respectively. Then, using the supernatant of recombinant *Bacillus subtilis* WB46 containing pP(aprE)-YdjM, they synthesized the enzyme using the resulting 7.2-fold increase in enzyme activity (Wang et al., 2019). Mechanism of synthesis & secretion of keratinase by *Bacillus subtilis* (Figure 4).

Efficient Production of a-*Monoglucosyl Hesperidin by Cyclodextrin Glucanotransferase From* Bacillus subtilis

In order to increase a target protein's secretory expression and secretion efficiency, it is necessary to screen each protein and determine its ideal signal peptide (Ji et al., 2023). A novel approach to logically maximizing the secretion signal peptide of exogenous proteins is offered by the investigation of signal peptides with high secretion efficiency (Akram et al., 2022). In several exogenous expression systems, the secretion of keratinase synthesis can be stimulated through systematic optimization of the ideal signal peptide, α-Monoglucosyl hesperidin exhibits a multitude of actions, making it a promising food additive (Su et al., 2020). In order to

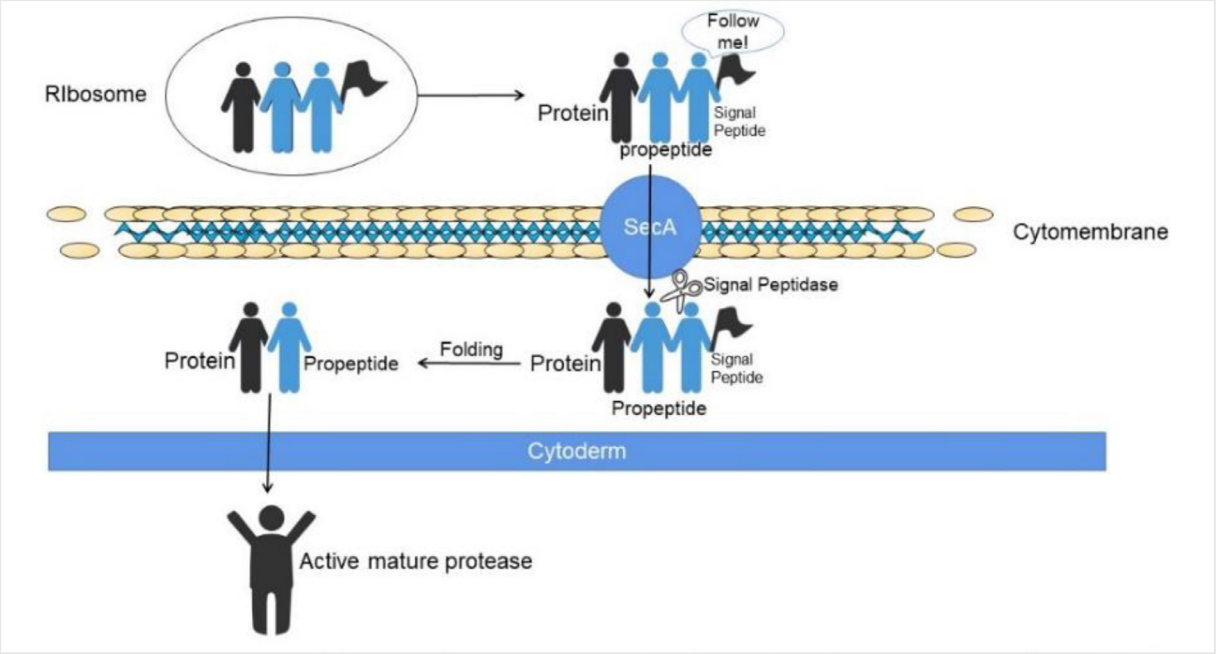

Figure. 4. Mechanism of synthesis & secretion of keratinase by *Bacillus subtilis*.

express cyclodextrin glucanotransferase (**CGTase**) from Bacillus sp. A2-5a, nonpathogenic *Bacillus subtilis* has been used as the host (Figure 4). To maximize B. subtilis CGTase transcription and secretion, promoters and signal peptides were examined (Zhou et al., 2023). The optimal signal peptide and promoter, according to optimization results, were YdjM and PaprE, respectively (Davis-Gardner et al., 2023; Zhou et al., 2023). Ultimately, the enzyme mediated synthesis yield of α-monoglucosyl hesperidin using the supernatant of the recombinant *B. subtilis WB800* harboring the plasmid pPaprE-YdjM produced the highest yield of 2.70 g/L, and the enzyme activity increased to 46.5 U/mL, which is 8.7 times that of the enzyme expressed from the strain containing pPHpaII-LipA. With recombinant CGTase, this is the highest degree of α-monoglucosyl hesperidin synthesis to date. This study explored a widely-applicable technique for producing α-monoglucosyl hesperidin at a larger scale (Zhou et al., 2023). CGTase expression had been optimized and improved through the medium components. Based on the findings, varying amounts of N and C sources will yield distinct values for the activity and biomass of β-CGTase. For CGTase expression, 10 g/L and 15 g/L of yeast and peptone extract were the optimal concentrations. Additionally, 15 g/L was the optimal glucose concentration for CGTase expression so the glucose concentration had been optimized (Duan et al., 2020). Additionally, the impact of metal ions on CGTase expression was examined. (Li et al., 2018). The findings demonstrated that although Mg^{2+}, Co^{2+}, and Cu^{2+} inhibited the secretion of CGTase, Fe^{3+} may increase the activity of CGTase (11.5 U/mL) and biomass of the strain. The CGTase secretion was unaffected by the metal ions Mn^{2+} and Ca^{2+} (Li et al., 2010; Sivapragasam and Abdullah 2015; Li et al., 2018). It is stopped growing the fermentation & the strain's growth on reaching 40th h of fermentation, the supernatant's CGTase production reached at optimum limit at 46.54U/mL (Wang et al., 2018; Zhou et al., 2023).

Based on the α-monoglucosyl hesperidin production data, hesperidin decreased over the first 2 h of the reaction while the concentration of α-monoglucosyl hesperidin rose dramatically. Following then, the α-monoglucosyl hesperidin concentration increased steadily and peaked at 2.70 g/L during the third hour of the reaction (Zhou et al., 2023). Hesperidin's conversion rate was 78.35%. After 3 h of processing, the concentration of α-monoglucosyl hesperidin finally stabilized. Hesperidin is the source of α-Monoglucosyl hesperidin, a flavanone glycoside (Kapoor et al., 2023; Zhou et al., 2023). It was observed that α-monoglucosyl hesperidin showed improved water solubility and the same biological activity as hesperidin in vivo (Uchiyama et al., 2011; Chen et al., 2023; Figueira et al., 2023). The α-monoglucosyl hesperidin is a potential chemical with good qualities that can be used in dietary supplements and food additives. Because of its great efficiency, safety, and environmental friendliness, enzymatic synthesis is a good option for α-monoglucosyl hesperidin production on a larger scale (Sandoval et al., 2020; Chen et al., 2023; Zhou et al., 2023). The only enzyme known to catalyze the conversion of hesperidin to α-monoglucosyl hesperidin was CGTase, which was isolated from *alkalophilic Bacillus* sp. A2-5a in a prior research (Kometani et al. 1994; Liu et al., 2022; Zhou et al., 2023). Furthermore, at alkaline conditions (pH 10.0−10.5), *Bacillus sp.* A2-5a's CGTase can sustain a high glycosylation activity, and hesperidin's solubility is also high at this pH

(Zhou et al., 2023). For this reason, the CGTase enzyme from the alkalophilic Bacillus sp. A2-5a was used to create α-monoglucosyl hesperidin. The benefits of genetically modified CGTases are numerous and include increased protein expression, reduced cost, and increased efficiency (Lin et al., 2023; Zhou et al., 2023). Enzyme engineering, for instance, allowed us to increase the *kcat*/KmA value of CGTase 6.43 times as compared to the wild type in our earlier research. The heterologous expression strategy was then used to produce the mutant (Chen et al., 2022; Zhou et al., 2023). The heterologous production of the *alkalophilic Bacillus* sp. A2-5a CGTase in *Bacillus subtilis,* however, has only been shown once (Dolya et al., 2023; Xu et al., 2023; Zhou et al., 2023). Mechanism of synthesis & secretion of keratinase by *Bacillus subtilis* as shown in Figure 4.

BIOENGINEERING OF KERATINASE SYSTEMS

Even though bacteria found in natural habitats possess remarkable enzymatic properties, application of molecular biology techniques might further improve the catabolic features and kinetic parameters with which they interact. An enzyme that has been genetically engineered can be synthesized, which opens the door to the possibility of using natural biocatalysts that are exceptionally efficient. The main steps for producing keratinase in a different organism are shown in Figure 5.

MOLECULAR TECHNIQUES TO ELEVATE THE PRODUCTION OF KERATINASE IN *E. COLI*

Latest studies on keratinases have been focused on isolating keratinases from different microbes and evaluating their expression after transfer into *E. Coli*. As *E. Coli* is the most used strain of microbes and is used as the host of expression of keratinases that comprises the various benefits as compared to the native host strain (Devi et al., 2023). At first the *E.coli* strain is used to optimize the recombinant keratinase productivity and expression, secondly the myriads vector may be used to control the recombinant keratinase induction, thirdly recombinant expression of keratinases may be justified through autoinducible or inductive constitutive promotors and fourth It is possible to use rational design to the cloned recombinant keratinase to enhance its properties (Jiang et al., 2017).

Keratinases obtained from the various beneficial microbes as *Deinococcus radiodurans* and *Meiothermus sp.,* (Jiang et al., 2017), *A. viridilutea* (Elhoul et al., 2016; Ben Elhoul et al., 2021), P. aeruginosa (Sharma and Gupta 2010), *B. pumilus* (Rajput et al., 2012), *B. amyloliquefaciens* (Zhang et al., 2016), *B. subtilis* (Gupta et al., 2017), *B. polyfermenticus* (Dong et al., 2017) and B. *licheniformis* (Nnolim et al., 2020a) had been successfully cloned and expressed in *E. Coli*. The reason for extensive use of *E. Coli* is the genetic flexibility so it is widely selected as a vector that is frequently used as a heterologous host for expressing the recombinant keratinases (Gupta et al., 2017).

The tagged protein form of recombinant keratinase, wherein the keratinase is coupled with a His-tag on its C-terminal, can be expressed through the use of pET vectors. The recombinant keratinase may be purified using Ni^{2+} affinity chromatography without experiencing misfolding events owing to the inclusion of His-tag (Wang et al., 2023a). However, if the high concentration of ionic or hydrophobic residues in the recombinant keratinase prevented it from being purified using Ni2+ affinity chromatography, then alternative purification techniques might be used (Lin et al., 2023).

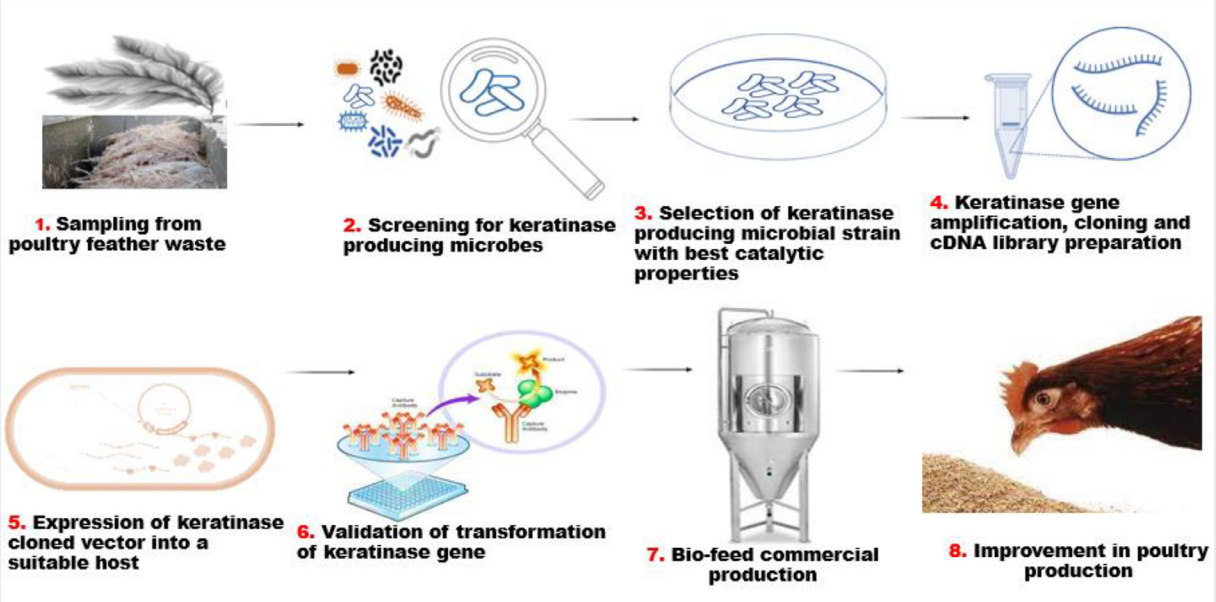

Figure 5. The main steps for producing keratinase in a different organism.

The recombinant keratinases' characteristics would also change as a result of the propeptides' varied folding routes. For example, in the recombinant *E. coli BL21* (DE3), the pro-sequences of 2 distinct keratinases, known as *KerB*Land, *KerB*P were switched, improving *KerB*L's physicochemical properties. It seemed likely that *KerB*L had adopted *KerB*P's physicochemical characteristics, suggesting that *KerB*L's morphological alterations as a result of propeptide swapping were comparable to those of *KerB*P (Gahatraj et al., 2023).

CONCLUSIONS AND FUTURE PROSPECTS

The biodegradation of massive poultry waste would prove a valuable protein source and a cost-effective recycling of keratinous waste products. There are good and effective microbe catalogs available for the degradation of such keratinous waste, which can hydrolyze poultry waste into peptides and amino acids along with free nitrogen. A cost-effective source of recycled protein that can be used again in poultry feed by using microbes. This type of recycled protein has been shown to be superior for the poultry industry and has been shown to improve the digestibility, growth and gut health of chickens. Large-scale methods to convert such wastes into bioconversion wastes using potent keratinolytic microbial strains could overcome the limitations of composting and effectively extend sustainable feeding resource for the poultry industry. *Lactobacillus* cloned keratinases, or proteolytic enzymes, are acid-resistant, just like other protease-resistant enzymes. They enhance acidity in the stomach, which is beneficial for the gastrointestinal tract. Poultry feathers, soya, and rapeseed keratin that are fermented using proteolytic enzymes are beneficial for the gut health of poultry. It offers the opportunity to replace expensive fish meal with low quality cheaper ingredients like feathers, wool, blood, and meat waste at a higher level, which can prove to be more cost-effective compared to fish meal. Engineered keratinases exhibit high activity and resistance to various conditions, and these biotechnologically engineered bacteria are a marvelous source of single cell digestible protein. These keratinolytic strains of microbes exhibit excellent activity in keratin waste like feathers, provide incredible utilization of potential keratin degraders, and will prove a definite source of biotechnological usage of various industrial and keratin hydrolysis processes. It can be an excellent solution for the limitations of the degradation of a massive poultry waste. They will be useful for recycling keratinaceous waste material in cost-effective and environmentally safe protein ingredients. Future research should be done to understand its keratinases, expression and mechanism of action in food-grade bacteria like Lactic acid bacteria.

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DISCLOSURES

The authors declared that there have no conflicts of interest.

REFERENCES

Abdelmoteleb, A., D. Gonzalez-Mendoza, and A. F. Roumia. 2023. Keratinases from *Streptomyces netropsis* and *Bacillus subtilis* and Their Potential Use in the Chicken Feather Degrading. Ferment. 9:96.

Abd El-Wahab, A., M. F. E. Ahmed, and J. Kamphues. 2022. Effect of dietary β-Mannanase addition on performance, pododermatitis, and intestinal morphology as well as digesta in broiler chickens: a pilot study. Ann. Anim. Sci. 22:1027–1039.

Aguilar Suárez, R., M. Antelo-Varela, and J. M.v. Dijl. 2021. Redirected stress responses in a genome-minimized 'midi Bacillus' strain with enhanced capacity for protein secretion. Msystems 6:1–19.

Akram, F., A. Aqeel, M. Shoaib, I. U. Haq, and F. I. Shah. 2022. Multifarious revolutionary aspects of microbial keratinases: an efficient green technology for future generation with prospective applications. Environ. Sci. Pollut. Res. 29:86913–86932.

Al Mamun, M. A. 2019. Bioprocess Development for Production of Proteases by *Bacillus Licheniformis*. Doctoral dissertation. University of Dhaka, Dhaka, Bangladesh.

Alvarez-Vera, M., J. Vázquez, D. Fernández, and R. Reinoso. 2022. Poultry Feathers and Offal Treatment by Using Beneficial Microorganisms. BioResources 17:64. Accesse on https://openurl.ebsco.com/EPDB%3Agcd%3A4%3A16440660/detailv2?sid=ebsco%3Aplink%3Ascholar&id=ebsco%3Agcd%3A155219299&crl=c.

Alwakeel, S. S., F. Ameen, H. Al Gwaiz, H. Sonbol, S. Alghamdi, Moharram, and O. A. Al-Bedak. 2021. Keratinases produced by Aspergillus stelliformis, Aspergillus sydowii, and Fusarium brachygibbosum isolated from human hair: Yield and activity. Journal of Fungi 7:471.

Ayanwale, B. A., E. C. Egwim, and E. Paul. 2023. Growth performance and meat quality of broiler chickens on diets containing Keratinase-treated and untreated feather meal-based diets. Acta Agric. Scand. A Anim.1–11, 2194310.

Ben Elhoul, M., N. Z. Jaouadi, and B. Jaouadi. 2021. Heterologous expression and purification of keratinase from *Actinomadura viridilutea DZ50*: feather biodegradation and animal hide dehairing bioprocesses. Environ. Sci. Pollut. Res. 28:9921–9934.

Benkiar, A., Z. J. Nadia, A. Badis, and B. Jaouadi. 2013. Biochemical and molecular characterization of a thermo-and detergent-stable alkaline serine keratinolytic protease from *Bacillus circulans* strain DZ100 for detergent formulations and feather-biodegradation process. Int. Biodeterior. Biodegradation. 83:129–138.

Berks, B. C., T. Palmer, and F. Sargent. 2003. The Tat protein translocation pathway and its role in microbial physiology. Adv Microb Physiol 47:187–254.

Beyenal, H., S. N. Chen, and Z. Lewandowski. 2003. The double substrate growth kinetics of *Pseudomonas aeruginosa*. Enzyme Microb. Technol. 32:92–98.

Bouacem, K., A. Bouanane-Darenfed, and B. Jaouadi. 2016. Novel serine keratinase from *Caldicoprobacter algeriensis* exhibiting outstanding hide dehairing abilities. Int J Biol Macromol 86:321–328.

Brandelli, A. 2008. Bacterial keratinases: useful enzymes for bioprocessing agroindustrial wastes and beyond. Food Bioproc Tech 1:105–116.

Brandelli, A., D. J. Daroit, and A. Riffel. 2010. Biochemical features of microbial keratinases and their production and applications. Appl Microbiol Biotechnol 85:1735–1750.

Brandelli, A., L. Sala, and S. J. Kalil. 2015. Microbial enzymes for bioconversion of poultry waste into added-value products. Food Res. Int. 73:3–12.

Brown, A. T. 2019. The Effect of Feed Additives on Male Broiler Performance. Theses and Dissertations. Mississippi State University, Mississippi.

Cai, M. Z., P. E. Kee, H. S. Ng, and P. T. Chen. 2022. Development of *Bacillus subtilis* self-inducible expression system for keratinase production using piggery wastewater. J Taiwan Inst Chem Eng 137:104218.

Cavello, I., B. Bezus, and S. Cavalitto. 2021. The keratinolytic bacteria *Bacillus cytotoxicus* as a source of novel proteases and feather protein hydrolysates with antioxidant activities. J Genet Eng Biotechnol 19:1–12.

Chen, H., J. Wang, Y. Liu, and X. Chen. 2023. Rational design of cyclodextrin glycosyltransferase with improved hesperidin glycosylation activity. Catalysts. 13:885.

Chen, H., S. Zhang, I. Park, and S. W. Kim. 2017. Impacts of energy feeds and supplemental protease on growth performance, nutrient digestibility, and gut health of pigs from 18 to 45 kg body weight. Anim Nutr 3:359–365.

Chen, J., S. Yang, S. Liang, F. Lu, K. Long, and X. J. B. Zhang. 2020. In vitro synergistic effects of three enzymes from *Bacillus subtilis CH-1* on keratin decomposition. 3 Biotech 10:1–7.

Chen, R., Y. Liu, S. Chen, M. Wang, Y. Zhu, T. Hu, and T. Xie. 2022. Protein engineering of a germacrene A synthase from *Lactuca sativa* and its application in high productivity of germacrene A in *Escherichia coli*. Front. Plant Sci. 13:932966.

Chen, X., C. Li, M. T. Bernards, and Y. He. 2021. Sequence-based peptide identification, generation, and property prediction with deep learning: a review. Mol. Syst. Des. Eng. 6:406–428.

Chitte, R., V. Nalawade, and S. Dey. 1999. Keratinolytic activity from the broth of a feather-degrading thermophilic *Streptomyces thermoviolaceus* strain SD8. Lett. Appl. Microbiol. 28:131–136.

Chojnacka, K., K. Mikula, G. Izydorczyk, and M. Korczyński. 2021. Innovative high digestibility protein feed materials reducing environmental impact through improved nitrogen-use efficiency in sustainable agriculture. J. Environ. Manage. 291:112693.

Cui, C., L. Sun, X. Chen, Y. Zhu, and B. Feng. 2023. Improving the biodegradation of chicken feathers by a *Bacillus licheniformis ZSZ6* mutant and application of hydrolysate. Int. Biodeterior. Biodegradation. 180:105597.

Datt, S., S. Das, M. A. Ansari, R. Sharma, T. Datt, and S. Bhattacharya. 2021. Gene expression profiling of protease and non-protease genes in *Trichophyton mentagrophytes* isolates from dermatophytosis patients by qRT-PCR analysis. Sci. Rep. 11:403.

Davis-Gardner, M. E., J. A. Weber, and M. R. Gardner. 2023. A strategy for high antibody expression with low anti-drug antibodies using AAV9 vectors. Front. Immunol. 14:1105617.

de Menezes, C. L. A., R. D. C. Santos, and R. da Silva. 2021. Industrial sustainability of microbial keratinases: production and potential applications. World J. Microbiol. Biotechnol 37:86.

Deniz, I., F. Zihnioglu, S. S. Öncel, E. E. Hames, and F. J. B. Vardar-Sukan. 2019. Production, purification and characterization of a proteolytic enzyme from *Streptomyces sp. 2M21*. Biocatal. Biotransformation. 37:377–387.

De Oliveira Martinez, J. P., G. Cai, M. Nachtschatt, and R. Speight. 2020. Challenges and opportunities in identifying and characterising keratinases for value-added peptide production. Catalysts 10:184.

de Paiva, D. P., S. S. A. de Oliveira, and S. S. de Oliveira. 2019. Keratinolytic activity of *Bacillus subtilis LFB-FIOCRUZ 1266* enhanced by whole-cell mutagenesis. Biotech 9:1–12.

Desai, P. N., N. Shrivastava, and H. Padh. 2010. Production of heterologous proteins in plants: strategies for optimal expression. Biotechnol. Adv. 28:427–435.

Devi, S., A. Chauhan, R. Bishist, and N. Sharma. 2023. Production, partial purification and efficacy of keratinase from *Bacillus halotolerans L2EN1* isolated from the poultry farm of Himachal Pradesh as a potential laundry additive. Biocatal. Biotransform. 41:222–242.

Dhanasingh, I., and S. H. Lee. 2019. Crystallization and preliminary X-ray diffraction analysis of Thioredoxin from the feather-degrading thermophile *Fervidobacterium islandicum AW-1*. Biodesign 7:47–51.

Dhiva, S., K. R. Ranjith, P. Prajisya, K. P. Sona, G. Narendrakumar, P. Prakash, R. E. Renitta, and A. V. Samrot. 2020. Optimization of keratinase production using Pseudomonas aeruginosa Su-1 having feather as substrate. Biointerface Res. Appl. Chem. 10:6540–6549.

Dolya, B., O. Hryhorieva, K. Sorochynska, and B. Ostash. 2023. Properties of multidrug-resistant mutants derived from heterologous expression chassis strain *Streptomyces albidoflavus J1074*. Microorg. 11:1176.

Dong, Y.-Z., W.-S. Chang, and Chen. Characterization and overexpression of a novel keratinase from *Bacillus polyfermenticus B4* in recombinant Bacillus subtilis. Bioresour. Bioprocess. 4:1–9.

Duan, M., Y. Wang, G. Yang, J. Li, and Y. Mao. 2020. High-level production of γ-cyclodextrin glycosyltransferase in recombinant *Escherichia coli BL21 (DE3)*: culture medium optimization, enzymatic properties characterization, and product specificity analysis. Ann. Microbiol 70:1–13.

Elhoul, M. B., N. Z. Jaouadi, H. Rekik, and B. Jaouadi. 2016. Biochemical and molecular characterization of new keratinoytic protease from *Actinomadura viridilutea DZ50*. Int. J. Biol. 92:299–315.

El-Refai, H. A., M. A. AbdelNaby, and A. A. Fattah. 2005. Improvement of the newly isolated *Bacillus pumilus FH9* keratinolytic activity. Process Biochem 40:2325–2332.

Fang, Z., J. Zhang, B. Liu, L. Jiang, G. Du, and J. Chen. 2014. Cloning, heterologous expression and characterization of two keratinases from *Stenotrophomonas maltophilia BBE11-1*. Process Biochem 49:647–654.

Figueira, O., V. Pereira, and P. C. Castilho. 2023. A two-step approach to orange peel waste valorization: consecutive extraction of pectin and hesperidin. Foods 12:3834.

Frain, K. M., C. Robinson, and J. M. van Dijl. 2019. Transport of folded proteins by the tat system. Protein 38:377–388.

Freudl, R. 2018. Signal peptides for recombinant protein secretion in bacterial expression systems. Microb Cell Fact 17:52.

Friedrich, I., H. Neubauer, A. Kuritsyn, and R. Daniel. 2023. Brevundimonas and Serratia as host systems for assessing associated environmental viromes and phage diversity by complementary approaches. Front. Microbiol. 14:1095850.

Fries-Craft, K., R. Arsenault, and E. Bobeck. 2023. Basal diet composition contributes to differential performance, intestinal health, and immunological responses to a microalgae-based feed ingredient in broiler chickens. Poult. Sci. 102:102235.

Fu, G., J. Liu, J. Li, B. Zhu, and D. Zhang. 2018. Systematic screening of optimal signal peptides for secretory production of heterologous proteins in *Bacillus subtilis*. Agric. Food Sci 66:13141–13151.

Gahatraj, I., A. Borah, P. Pandey, and S. Kumar. 2023. Current progress and biotechnological applications of microbial keratinases. J Pure Appl Microbiol 17:732–748.

Gegeckas, A., R. Gudiukaitė, J. Debski, and D. Citavicius. 2015. Keratinous waste decomposition and peptide production by keratinase from *Geobacillus stearothermophilus AD-11*. Int. J. Biol. Macromol. 75:158–165.

Ghayour-Najafabadi, P., H. Khosravinia, A. Gheisari, A. Azarfar, and M. Khanahmadi. 2018. Productive performance, nutrient digestibility and intestinal morphometry in broiler chickens fed corn or wheat-based diets supplemented with bacterial-or fungal-originated xylanase. Ital. J. Anim. Sci. 17:165–174.

Golunski, S. M., L. Sala, M. F. Silva, R. M. Dallago, J. Mulinari, A. J. Mossi, A. Brandelli, S. J. Kalil, M. Di Luccio, and H. Treichel. 2016. Interference of salts used on aqueous two-phase systems on the quantification of total proteins. Int. J. Biol. Macromol. 83:30–33.

Gong, J. S., J. P. Ye, L. Y. Tao, and J. S. Shi. 2020. Efficient keratinase expression via promoter engineering strategies for degradation of feather wastes. Enzyme Microb. Technol. 137:109550.

González, V., M. J. Vargas-Straube, and B. Cámara. 2020. Enzyme bioprospection of marine-derived actinobacteria from the chilean coast and new insight in the mechanism of keratin degradation in *Streptomyces sp. G11C*. Mar. Drugs. 18:537.

Grazziotin, A., F. A. Pimentel, E. V. De Jong, and A. Brandelli. 2006. Nutritional improvement of feather protein by treatment with microbial keratinase. Anim. Feed Sci. Technol 126:135–144.

Greenwald, J., and R. Riek. 2012. On the possible amyloid origin of protein folds. J. Mol. Biol 421:417–426.
Gupta, R., Q. Beg, and P. Lorenz. 2002. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl. Microbiol. Biotechnol. 59:15–32.
Gupta, S., S. Sharma, A. Aich, and S. Kalia. 2023. Chicken feather waste hydrolysate as a potential biofertilizer for environmental sustainability in organic agriculture management. Waste Biomass Valorization 14:2783–2799.
Gupta, S., P. Tewatia, J. Misri, and R. Singh. 2017. Molecular modeling of cloned *Bacillus subtilis* keratinase and its insinuation in psoriasis treatment using docking studies. Indian J. Microbiol. 57:485–491.
Hou, H. J., J. S. Gong, H. Li, and J. S. Shi. 2019. Phospholipase D engineering for improving the biocatalytic synthesis of phosphatidylserine. Bioprocess Biosyst Eng. 42:1185–1194.
Huang, C., D. Ma, J. Zang, B. Zhang, and S. Zhang. 2018. Effect of keratinase on ileal amino acid digestibility in five feedstuffs fed to growing pigs. Asian-Australas. J. Anim. Sci. 31:1946.
Huang, M., R. Chen, and G. Ren. 2017. Secretory expression and purification of *Bacillus licheniformis* keratinase in insect cells. PLoS One 12:e0183764.
Huang, Y., M. Łężyk, F.-A. Herbst, P. K. Busk, and L. Lange. 2020. Novel keratinolytic enzymes, discovered from a talented and efficient bacterial keratin degrader. Sci. Rep. 10:10033.
Huyan, L., A. Kumar, M. Manafi, and E. A. Soumeh. 2022. Effects of protease supplementation on growth performance, organ development, gut morphology, and microbial profile of broiler chicken. Acta Agric Scand A Anim Sci 71:40–50.
Ismail, A., and R. M. Illias. 2017. Site-saturation mutagenesis of mutant L-asparaginase II signal peptide hydrophobic region for improved excretion of cyclodextrin glucanotransferase. J Ind Microbiol Biotechnol 44:1627–1641.
Jagadeesan, Y., S. Meenakshisundaram, and A. Balaiah. 2020. Sustainable production, biochemical and molecular characterization of thermo-and-solvent stable alkaline serine keratinase from novel *Bacillus pumilus AR57* for promising poultry solid waste management. Int. J. Biol. Macromol. 163:135–146.
Jayalakshmi, T., P. Krishnamoorthy, G. Ramesh, and P. Sivamani. 2010. Isolation and screening of a feather-degrading keratinolytic actinomycetes from Actinomyces sp. Am. J. Sci. 6:45–48.
Ji, X., Z. Peng, J. Song, G. Zhang, and J. Zhang. 2023. Fusion of substrate-binding domains enhances the catalytic capacity of keratinases and promotes enzymatic conversion of feather waste. J. Agric. Food Chem. 71:11579–11586.
Jiang, S., J. Wang, X. Liu, Y. Liu, and M. Lin. 2017. DrwH, a novel WHy domain-containing hydrophobic LEA5C protein from *Deinococcus radiodurans*, protects enzymatic activity under oxidative stress. Sci. Rep 7:9281.
Jin, H. S., S. Y. Park, K. Kim, and D. W. Lee. 2017. Development of a keratinase activity assay using recombinant chicken feather keratin substrates. PloS one 12:e0172712.
Kang, D., J. Herschend, W. A. Al-Soud, and S. J. Sørensen. 2018. Enrichment and characterization of an environmental microbial consortium displaying efficient keratinolytic activity. Bioresour. Technol. 270:303–310.
Kang, Q., and D. Zhang. 2020. Principle and potential applications of the non-classical protein secretory pathway in bacteria. Appl. Microbiol. Biotechnol. 104:953–965.
Kapoor, M. P., M. Moriwaki, K. Uguri, and A. Abe. 2023. Improved bioavailability of hesperetin 7-O-glucoside inclusion complex with β-cyclodextrin in Sprague-Dawley rats and healthy humans. J. Funct. Foods 107:105708.
Kawase, T., F. Miura, A. Debnath, K. Imakura, and S. I. Miyoshi. 2018. Functional analysis of N-terminal propeptide in the precursor of Vibrio vulnificus metalloprotease by using cell-free translational system. Protein Expr. Purif. 149:13–16.
Kayalvizhi, N., and P. Gunasekaran. 2008. Production and characterization of a low-molecular-weight bacteriocin from Bacillus licheniformis MKU3. Lett. Appl. Microbiol. 47:600–607.
Khedmati, E., S. J. Hashemi-Hazaveh, R. Kachuei, M. Bayat, and K. Amini. 2023. Identification of subtilisin genes as a potential virulence factor in *Trichophyton mentagrophytes* isolated from human and bovine dermatophytosis lesions in Iran. Comp. Clin. Path. 32:217–224.
Kieliszek, M., K. Pobiega, K. Piwowarek, and A. M. Kot. 2021. Characteristics of the proteolytic enzymes produced by lactic acid bacteria. Mol 26:1858.
Kojima, M., M. Kanai, M. Tominaga, S. Kitazume, A. Inoue, and K. J. E. Horikoshi. 2006. Isolation and characterization of a feather-degrading enzyme from *Bacillus pseudofirmus FA30-01*. Extremophiles 10:229–235.
Kometani, T., Y. Terada, T. Nishimura, H. Takii, and S. Okada. 1994. Transglycosylation to hesperidin by cyclodextrin glucanotransferase from an alkalophilic Bacillus species in alkaline pH and properties of hesperidin glycosides. Biosci. Biotechnol. Biochem. 58:1990–1994.
Laboissière, M., M. A. da Costa, and J. H. Stringhini. 2020. Feather and blood meal at different processing degrees in broiler pre-starter and starter diets. Braz. J.Anim. Sci. 49:1–11.
Ladha, J. K., M. L. Jat, C. M. Stirling, and B. Gerard. 2020. Achieving the sustainable development goals in agriculture: the crucial role of nitrogen in cereal-based systems. Adv. Agron. 163:39–116.
Lai, Y., X. Wu, X. Zheng, W. Li, and L. Wang. 2023. Insights into the keratin efficient degradation mechanism mediated by *Bacillus sp. CN2* based on integrating functional degradomics. Biotechnol. Biofuels Bioproducts 16:59.
Latka, C., J. Bikshapathi, P. Aggarwal, and B. Taneja. 2020. Structural analysis of Sub16 sedolisin of *Trichophyton rubrum* reveals a flexible nature of its pro domain. bioRxiv 2020, doi:10.1101/2020.01.28.922815.
Lee, H., D. Suh, J. Hwang, and H. J. A.b. Suh. 2002. Characterization of a kerationlytic metalloprotease from *Bacillus sp. SCB-3*. Appl. Biochem. Biotechnol. 97:123–133.
Lee, T. Y., Y. S. Lee, R. H. Yeh, K. H. Chen, and K. L. Chen. 2022. *Bacillus amyloliquefaciens CU33* fermented feather meal-soybean meal product improves the intestinal morphology to promote the growth performance of broilers. Poult. Sci. 101:102027.
Li, C., S. Chen, Z. Gu, Y. Hong, L. Cheng, and Z. Li. 2018. Enhancement of α-CGTase thermostability with the addition of calcium or barium ions. Food Biosci. 26:139–144.
Li, J., D. Che, Z. Yu, L. Zhao, and R. Zhang. 2013. Improvement of expression level of keratinase Sfp2 from *Streptomyces fradiae* by site-directed mutagenesis of its N-terminal pro-sequence. Biotechnol. Lett. 35:743–749.
Li, Q. 2022. Perspectives on converting keratin-containing wastes into biofertilizers for sustainable agriculture. Front. Microbiol. 13:918262.
Li, Y., B. Guo, Z. Wu, W. Wang, C. Li, G. Liu, and H. J. A. Cai. 2020. Effects of fermented soybean meal supplementation on the growth performance and cecal microbiota community of broiler chickens. Animals 10:1098.
Li, Z., B. Li, Z. Gu, G. Du, J. Wu, and J. Chen. 2010. Extracellular expression and biochemical characterization of α-cyclodextrin glycosyltransferase from *Paenibacillus macerans*. Carbohydr. Res. 345:886–892.
Lin, W., Q. Wang, R. Han, J. Zhou, G. Xu, and Y. Ni. 2023. Engineering of methionine adenosyltransferase reveals key roles of electrostatic interactions in enhanced catalytic activity. Biotechnol. Appl. Biochem. 1–14, doi:10.1007/s12010-023-04676-7.
Liu, B., J. Zhang, Z. Fang, G. Du, J. Chen, and X. Liao. 2014. Functional analysis of the C-terminal propeptide of keratinase from *Bacillus licheniformis BBE11-1* and its effect on the production of keratinase in Bacillus subtilis. Process Biochem 49:1538–1542.
Liu, L., H. Yang, J. Li, G. Du, and J. Chen. 2013. How to achieve high-level expression of microbial enzymes: strategies and perspectives. Bioengineered 4:212–223.
Liu, Z., J. Liu, L. Ren, J. Wu, and S. Chen. 2022. Preparation of high-quality resistant dextrin through pyrodextrin by a multienzyme complex. Food Biosci. 47:10170.
Liya, S. M., M. Umesh, A. Nag, and K. Brindhadevi. 2023. Optimized production of keratinolytic proteases from *Bacillus tropicus LS27* and its application as a sustainable alternative for dehairing, destaining and metal recovery. Environ. Res. 221:115283.

Low, K. O., N. Muhammad Mahadi, and R. Md. Illias. 2013. Optimisation of signal peptide for recombinant protein secretion in bacterial hosts. Appl. Microbiol. Biotechnol. 97:3811–3826.

Lu, Y., Y. L.v X.Tan, Y. C. G.Yang, and Q. He. 2020. Physicochemical properties and microbial community dynamics during Chinese horse bean-chili-paste fermentation, revealed by culture-dependent and culture-independent approaches. Food Microbiology 85:103309.

Luong, V. B., and C. Payne. 1977. Hydrolysed feather protein as a source of amino acids for laying hens. Br. Poult. Sci. 18:523–526.

Mamangkey, J., D. Suryanto, E. Munir, and A. Z. Mustopa. 2020. Keratinase activity of a newly keratinolytic bacteria, *Azotobacter chroococcum B4*. J Pure Appl Microbiol 14:1203–1211.

Mechri, S., K. Bouacem, N. Z. Jaouadi, and B. Jaouadi. 2019. Identification of a novel protease from the thermophilic *Anoxybacillus kamchatkensis M1V* and its application as laundry detergent additive. Extremophiles 23:687–706.

Mendel, S., and C. Robinson. 2007. Targeting of proteins by the twin-arginine translocation system in bacteria and chloroplasts. The Enzymes 25:69–91.

Moridshahi, R., M. R. Sharifmoghadam, and M. Bahreini. 2021. Biodegradation of keratinous wastes by alkaline-keratinase produced from *Bacillus Tequilensis Bk206* and optimizing the cultural conditions for increased keratinolytic activity. J. Appl. Biol. Sci. 15:185–195.

Muller, A., C. Schader, and U. Niggli. 2017. Strategies for feeding the world more sustainably with organic agriculture. Nat. Commun. 8:1–13.

Naseer, A., M. Fayyaz, M. Sharif, and A. Sarfraz. 2022. Isolation and molecular characterization of keratinase producing Bacillus species from soil. Biomed. Lett. 8:117–122.

Nnolim, N. E., L. Mpaka, A. I. Okoh, and U. Nwodo. 2020a. Biochemical and molecular characterization of a thermostable alkaline metallo-keratinase from *Bacillus sp. Nnolim-K1*. Microorganisms 8:1304.

Nnolim, N. E., L. Mpaka, A. I. Okoh, and U. Nwodo. 2020b. Microbial keratinase: next generation green catalyst and prospective applications. Front. Microbiol. 11:580164.

Nnolim, N. E., and U. U. Nwodo. 2021. Microbial keratinase and the bio-economy: a three-decade meta-analysis of research exploit. AMB Express 11:1–16.

Odetallah, N., J. Wang, J. Garlich, and J. Shih. 2003. Keratinase in starter diets improves growth of broiler chicks. Poult. Sci. 82:664–670.

Ossai, I. C., F. S. Hamid, and A. Hassan. 2022. Valorisation of keratinous wastes: a sustainable approach towards a circular economy. J. Waste Manag. 151:81–104.

Oyeagu, C. E., V. Mlambo, and F. B. Lewu. 2023. Histomorphometric traits, microbiota, nutrient digestibility, growth performance, carcass traits and meat quality parameters of chickens fed diets supplemented with different levels of Bacillus protease. J. Appl. Anim Res. 51:137–155.

Papadopoulos, M. C., A. R. El Boushy, and E. H. Ketelaars. 1986. Effects of processing time and moisture content on amino acid composition and nitrogen characteristics of feather meal. Anim. Feed Sci. Technol. 14:279–290.

Parinayawanich, S., D. Sittipol, and N. Jongruja. 2021. Application of recombinant hyperthermostable keratinase for degradation of chicken feather waste. Biocatal. Agric. Biotechnol. 36:102146.

Pei, X. D., F. Li, S. Y. Yue, X. N. Huang, T. T. Gao, D. Q. Jiao, and C. H. Wang. 2023. Production and characterization of novel thermo-and organic solvent−stable keratinase and aminopeptidase from *Pseudomonas Aeruginosa 4−3* for effective poultry feather degradation. Environ. Sci. Pollut. Res. 30:2480–2493.

Peng, Z., J. Zhang, Y. Song, R. Guo, G. Du, and J. Chen. 2021. Engineered pro-peptide enhances the catalytic activity of keratinase to improve the conversion ability of feather waste. Biotechnol. Bioeng. 118:2559–2571.

Peydayesh, M., M. Bagnani, W. L. Soon, and R. Mezzenga. 2022. Turning food protein waste into sustainable technologies. Chem. Rev. 123:2112–2154.

Pilgaard, B., C. Wilkens, F.-A. Herbst, M. Vuillemin, and L. Lange. 2019. Proteomic enzyme analysis of the marine fungus *Paradendryphiella salina* reveals alginate lyase as a minimal adaptation strategy for brown algae degradation. Sci. Rep. 9:12338.

Qiu, J., K. Barrett, C. Wilkens, and A. S. Meyer. 2022. Bioinformatics based discovery of new keratinases in protease family M36. N Biotechnol 68:19–27.

Qiu, J., C. Wilkens, K. Barrett, and A. S. Meyer. 2020. Microbial enzymes catalyzing keratin degradation: Classification, structure, function. Biotechnol. Adv. 44:107607.

Radha, S., and P. Gunasekaran. 2007. Cloning and expression of keratinase gene in *Bacillus Megaterium* and optimization of fermentation conditions for the production of keratinase by recombinant strain. J. Appl. Microbiol. 103:1301–1310.

Radha, S., and P. Gunasekaran. 2009. Purification and characterization of keratinase from recombinant Pichia and Bacillus strains. Protein Expr. Purif. 64:24–31.

Rahimnahal, S., A. Meimandipour, and Y. Mohammadi. 2023. Biochemical and molecular characterization of novel keratinolytic protease from *Bacillus Licheniformis (Krlr1)*. Front. Microbiol. 14:1132760.

Rahman, F. A. 2023. Zinc binding and chelating compounds as inhibitors of bacterial metalloproteases and human matrix metalloproteases. Molecules 27:56.

Rajput, R., and R. Gupta. 2013. Thermostable keratinase from *Bacillus pumilus KS12*: production, chitin crosslinking and degradation of Sup35NM aggregates. Biores. tech. 133:118–126.

Rajput, R., E. Tiwary, R. Sharma, and R. Gupta. 2012. Swapping of pro-sequences between keratinases of *Bacillus licheniformis* and *Bacillus pumilus*: altered substrate specificity and thermostability. Enzyme Microb. Technol. 51:131–138.

Ramalingum, N., P. Bhagwat, K. Permaul, and S. Pillai. 2022. Production, characterization, and application of *Pseudomonas aeruginosa S-04* keratinase for feather utilization. Biomass Convers. Biorefin. 1–13, doi:10.1007/s13399-022-03218-1.

Ramezani, A., E. M. Maymand, and A. Ghaderi. 2017. Improving Pertuzumab production by gene optimization and proper signal peptide selection. Protein Expr. Purif 135:24–32.

Rao, S. R., T. Srilatha, M. V. L. N. Raju, and B. Prakash. 2020. Effect of dietary supplementation of microbial protease on performance, egg quality and nitrogen retention in white leghorn layers. Anim. Prod. Sci. 61:139–145.

Ren, Y., H. Luo, H. Huang, N. Hakulinen, Y. Wang, Y. Wang, and B. Yao. 2020. Improving the catalytic performance of Proteinase K from *Parengyodontium album* for use in feather degradation. Int. J. Biol. Macromol. 154:1586–1595.

Riffel, A., S. Ortolan, and A. Brandelli. 2003. De-hairing activity of extracellular proteases produced by keratinolytic bacteria. J. Chem. Technol. Biotechnol. 78:855–859.

Ruiz-Villafán, B., R. Rodríguez-Sanoja, and S. Sánchez. 2023. Useful microbial enzymes—an introduction. Pages 11−23 in Biotechnology of Microbial Enzymes. Microb. Biotechnol. Academic Press, Cambridge, MA.

Salmanian, M., M. Shams Shargh, A. Yamchi, and M. H. Mohammadi Ghasem Abadi. 2022. Effect of grain type and phytase enzyme on growth performance, cecum microbial population, carcass and bone characteristics of broiler chickens. Am. J. Anim. Vet. Sci. 13:8–18.

Sandoval, V., H. Sanz-Lamora, G. Arias, and J. Relat. 2020. Metabolic impact of flavonoids consumption in obesity: From central to peripheral. Nutri 12:2393.

Schaller, A., A. Stintzi, S. Rivas, A. B.Vartapetian, and P. Vera. 2018. From structure to function−a family portrait of plant subtilases. New Phytol 218:901–915.

Schwabe, M., S. Griep, H. Schmidtberg, R. Plarre, and K. Brinkrolf. 2021. Next-generation sequencing analysis of the *Tineola bisselliella* larval gut transcriptome reveals candidate enzymes for keratin digestion. Genes 12:1113.

Sharma, I., P. Gupta, and N. Kango. 2023. Synthesis and characterization of keratinase laden green synthesized silver nanoparticles for valorization of feather keratin. Sci. Rep. 13:11608.

Sharma, I., and N. Kango. 2021. Production and characterization of keratinase by *Ochrobactrum intermedium* for feather keratin utilization. Int. J. Biol. Macromol. 166:1046–1056.

Sharma, R., and R. Gupta. 2010. Extracellular expression of keratinase Ker P from *Pseudomonas aeruginosa* in *E. coli*. Biotechnol. Lett. 32:1863–1868.

Sharma, S., S. Vaid, B. Bhat, S. Singh, and B. K. Bajaj. 2019. Thermostable enzymes for industrial biotechnology. Pages 469−495 in Advances in enzyme technology. Elsevier.

Shen, N., M. Yang, C. Xie, H. Zhang, and M. Jiang. 2022. Isolation and identification of a feather degrading *Bacillus tropicus* strain Gxun-17 from marine environment and its enzyme characteristics. BMC Biotechnol 22:11.

Sivapragasam, M., and N. Abdullah. 2015. Recovery of cyclodextrin glucanotransferase (cgtase) using immobilized metal chelating affinity chromatography. Braz. J. Chem. Eng. 32:43–52.

Solanki, P., C. Putatunda, A. Kumar, R. Bhatia, and A. Walia. 2021. Microbial proteases: ubiquitous enzymes with innumerable uses. 3 Biotech 11:428.

Song, C., K. H. Gardner, S. J. Klein, S. P. Souza, and W. Mo. 2018. Cradle-to-grave greenhouse gas emissions from dams in the United States of America. Renew. Sust. Energ. Rev. 90:945–956.

Song, P., Z. Xue, W. Shuhua, X. We, W. Fei, and W. Feng. 2023. Microbial proteases and their applications. Microb.produts 14:1236368.

Song, Y., J. M. Nikoloff, and D. Zhan. 2015. Improving protein production on the level of regulation of both expression and secretion pathways in *Bacillus subtilis*. J. Microbiol. Biotechnol. 25:963–977.

Soomro, R. N., J. Yao, M. A. Gola, A. Ali, M. N. Htet, and S. A. Soomro. 2017. Beneficial aspects of feeding soybean meal (SBM) fermented with fungal and bacterial actions in broiler chickens. Am J Biol Life Sci 5:21–29.

Stühmeier-Niehe, C., L. Lass, M. Brocksieper, P. Chanos, and C. Hertel. 2023. Pre-treatment of starter cultures with mild pulsed electric fields influences the characteristics of set yogurt. Foods 12:442.

Su, C., J. S. Gong, J. Qin, H. Li, and J. S. Shi. 2020. The tale of a versatile enzyme: molecular insights into keratinase for its industrial dissemination. Biotechnol. Adv. 45:107655.

Su, C., J. S. Gong, Y. X. Sun, and J. S. Shi. 2019. Combining pro-peptide engineering and multisite saturation mutagenesis to improve the catalytic potential of keratinase. ACS Synth. Biol. 8:425–433.

Su, C., J. S. Gong, Z. X. Wu, and Z. H. Xu. 2023. Development of a growth-dependent system to regulate cell growth and keratinase production in *B. subtilis*. J. Agric. Food Chem. 71:2421–2429.

Sun, H., D. Chen, H. Cai, W. Chang, Z. Wang, G. Liu, and Z. J. A. Chen. 2022. Effects of fermenting the plant fraction of a complete feed on the growth performance, nutrient utilization, antioxidant functions, meat quality, and intestinal microbiota of broilers. Animals 12:2870.

Sypka, M., I. Jodłowska, and A. M. J. B. Białkowska. 2021. Keratinases as versatile enzymatic tools for sustainable development. Biomolecules 11:1900.

Tamreihao, K., S. Mukherjee, R. Khunjamayum, L. J. Devi, R. S. Asem, and D. S. Ningthoujam. 2019. Feather degradation by keratinolytic bacteria and biofertilizing potential for sustainable agricultural production. J. Basic Microbiol. 59:4–13.

Thomas, C., and R. Tampé. 2018. Multifaceted structures and mechanisms of ABC transport systems in health and disease. Curr. Opin. Struct. Biol. 51:116–128.

Tian, J., X. Long, Y. Tian, and B. Shi. 2019. Enhanced extracellular recombinant keratinase activity in *Bacillus subtilis SCK6* through signal peptide optimization and site-directed mutagenesis. RSC Adv. 9:33337–33344.

Timorshina, S., E. Popova, V. Kreyer, N. Baranova, and A. Osmolovskiy. 2022. Keratinolytic Properties of *Aspergillus clavatus* Promising for Biodegradation. Int. J. Environ. Res. Public Health. 19:13939.

Tjalsma, H., H. Antelman, and J. M. van Dijl. 2004. Proteomics of protein secretion by *Bacillus subtilis*: separating the "secrets" of the secretome. Microbiol. Mol. Biol. 68:207–233.

Tjalsma, H., A. Bolhuis, J. D. Jongbloed, and J. M. van Dijl. 2000. Signal peptide-dependent protein transport in *Bacillus subtilis*: a genome-based survey of the secretome. Microbiol. Mol. Biol. 64:515–547.

Tolibia, S. E. M., A. D. Pacheco, and V. E. López y López. 2023. Engineering of global transcription factors in Bacillus, a genetic tool for increasing product yields: a bioprocess overview. World J. Microbiol. Biotechnol. 39:12.

Tork, S., M. M. Aly, and L. Nawar. 2010. Biochemical and molecular characterization of a new local keratinase producing *Pseudomomanas sp., MS21*. Asian J. Biotechnol. 2:1–13.

Uchiyama, H., Y. Tozuka, F. Asamoto, and H. Takeuchi. 2011. α-Glucosyl hesperidin induced an improvement in the bioavailability of pranlukast hemihydrate using high-pressure homogenization. Int. J. Pharm. 410:114–117.

Vidmar, B., and M. Vodovnik. 2018. Microbial keratinases: enzymes with promising biotechnological applications. Food Technol. Biotechnol. 56:312–328.

Walker, K. L., A. S. Jones, and C. Robinson. 2015. The Tat pathway as a biotechnological tool for the expression and export of heterologous proteins in *Escherichia coli*. Pharm. Bioprocess. 3:387–396.

Wang, D., X. S. Piao, Z. K. Zeng, T. Lu, and S. W. Kim. 2011. Effects of keratinase on performance, nutrient utilization, intestinal morphology, intestinal ecology and inflammatory response of weaned piglets fed diets with different levels of crude protein. Asian-australas. J. Anim. Sci. 24:1718–1728.

Wang, J., and J. Shih. 1999. Fermentation production of keratinase from Bacillus licheniformis PWD-1 and a recombinant *B. subtilis FDB-29*. Journal of Industrial J. Microbiol. Biotechnol. 22:608–616.

Wang, J. J., K. Rojanatavorn, and J. C. Shih. 2004. Increased production of Bacillus keratinase by chromosomal integration of multiple copies of the kerA gene. Biotechnol. Bioeng. 87:459–464.

Wang, L., S. Chen, and J. Wu. 2018. Cyclodextrin enhanced the soluble expression of *Bacillus clarkii* γ-CGTase in *Escherichia coli*. BMC Biotechnol 18:1–9.

Wang, L., Y. Qian, Y. Cao, Y. Huang, Z. Chang, and H. Huang. 2017. Production and characterization of keratinolytic proteases by a chicken feather-degrading thermophilic strain, *Thermoactinomyces sp. YT06*. J Microbiol Biotechno l27:2190–2198.

Wang, X., Y. Nie, and Y. Xu. 2019. Industrially produced pullulanases with thermostability: discovery, engineering, and heterologous expression. Bioresour. Technol. 278:360–371.

Wang, X., Y. Tang, J. Xu, and Q. Li. 2023a. The N-terminus of Spt16 anchors FACT to MCM2−7 for parental histone recycling. Nucleic Acids Res. 51:11549–11567.

Wang, X., Z. Yu, W. Liu, H. Tang, D. Yi, and M. Wei. 2021. Recent progress on MHC-I epitope prediction in tumor immunotherapy. Am. J. Cancer Res. 11:2401.

Wang, Z., Y. Chen, M. Yan, K. Li, and H. Chen. 2023. Research progress on the degradation mechanism and modification of keratinase. Appl. Microbiol. Biotechnol. 107:1003–1017.

Watts, A., S. Sankaranarayanan, A. Watts, and R. K. Raipuria. 2021. Optimizing protein expression in heterologous system: strategies and tools. Meta Gene 29:100899.

Weihang, W. A. N. G., L. U. O. Xinge, L. I. N. Tao, and L. I. Qin. 2023. Research progress of keratin degradation. Acta Ecol. Sin. 14:458–466.

Wu, W. S. M.a., R. Chen, Y. Huang, and Y. Deng. 2022. Genome-wide analysis of keratinibaculum paraultunense strain KD-1 T and its key genes and metabolic pathways involved in the anaerobic degradation of feather keratin. Arch. Microbiol. 204:634.

Xiang, M., Q. Kang, and D. Zhang. 2020. Advances on systems metabolic engineering of *Bacillus subtilis* as a chassis cell. Synth. Syst. Biotechnol. 5:245–251.

Xu, S., S. Gao, and Y. An. 2023. Research progress of engineering microbial cell factories for pigment production. Biotechnol. Adv. 10:108150.

Yahaya, R. S. R., Y. M. Normi, L. Y. Phang, and S. Sabri. 2021. Molecular strategies to increase keratinase production in heterologous expression systems for industrial applications. Appl. Microbiol. Biotechnol. 105:3955–3969.

Yong, B., X. Fei, H. Shao, P. Xu, Y. Hu, W. Ni, and H. Feng. 2020. Recombinant expression and biochemical characterization of a novel keratinase BsKER71 from feather degrading bacterium *Bacillus subtilis S1-4*. AMB Express. 10:1–10.

Yu, C., X. Huang, H. Chen, H. C. J. Godfray, and J. Taylor. 2019. Managing nitrogen to restore water quality in China. Nature. 567:516–520.

Zhang, N., D. Yang, J. R. Kendall, R. Borriss, and R. Zhang. 2016. Comparative genomic analysis of *Bacillus amyloliquefaciens* and *Bacillus subtilis* reveals evolutional traits for adaptation to plant-associated habitats. Front. Microbiol. 7:2039.

Zhang, R.-X., J.-S. Gong, C. Su, J. Qin, and Z.-H. Xu. 2020. Recombinant expression and molecular engineering of the keratinase from *Brevibacillus parabrevis* for dehairing performance. J. Biotech. 320:57–65.

Zheng, M., Y. Bai, Y. Sun, Q. C. J.An, and T. J. A. Zhang. 2023. Effects of different proteases on protein digestion in vitro and in vivo and growth performance of broilers fed corn−soybean meal diets. Animals 13:1746.

Zhou, J., Y. Shi, J. Fang, T. Gan, and X. Chen. 2023. Efficient production of α-monoglucosyl hesperidin by cyclodextrin glucanotransferase from *Bacillus subtilis*. Appl. Microbiol. Biotechnol. 102:1–11.

Zhou, L., X. Xie, T. Wu, and W. Zou. 2020. Compound enzymatic hydrolysis of feather waste to improve the nutritional value. Biomass Convers. Biorefin. 12:1–12.

Zhu, X., L. Tao, H. Liu, and G. J. P. S. Yang. 2023. Effects of fermented feed on growth performance, immune organ indices, serum biochemical parameters, cecal odorous compound production, and the microbiota community in broilers. Poult. Sci. 102:102629.