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Physiology of keratin waste biodegradation and keratinase production by mycelial fungi: a review

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Abstract

The ability to degrade keratin is widespread among all groups of microorganisms. From an environmental and economic perspective, the biodegradation of keratinous wastes by microorganisms and their keratinolytic enzymes is considered the most effective alternative for their processing and valorization. Moreover, the use of keratinases to deconstruct keratin waste is a promising approach for manufacturing animal feed, plant fertilizers, cosmetics, pharmaceuticals, tannery and leather, detergents, and bioplastics. As a result, the demand for these enzymes has increased significantly in recent years, requiring large quantities of low-cost enzymes to meet industrial needs. Ascomycota and Basidiomycota fungi are considered potential producers of keratinases in submerged and solid-state fermentations of keratinous waste materials. To increase keratinase yields, various approaches and strategies, such as the use of cheap materials as growth substrates, optimization of fermentation media and cultivation conditions, and development of improved bioprocess technologies, have been widely exploited. However, compared to bacteria, understanding the regulatory mechanisms of keratin-degrading enzyme production in filamentous fungi is still in its infancy. This review provides the first systematic analysis of the cultural and physicochemical factors modulating the keratinolytic activity of Ascomycota and Basidiomycota fungi. The need for a thorough study of the microbial diversity of keratinolytic fungi to identify fungi that overproduce keratinase is emphasized. Understanding key aspects of the physiological regulation of target enzyme synthesis by each specific fungal strain is crucial for increasing keratinase yield.

Keywords

Chicken feathers, Fermentation, Keratinases, Mycelial fungi, Regulation.

Introduction

The poultry, meat processing, leather, and wool textile industries produce large amounts of keratin waste annually, and its disposal through incineration, landfilling, chemical hydrolysis, and carbonization causes serious environmental

problems and requires substantial energy expenditure (Nad et al., 2024; JL and Dhanasingh, 2025). Various hydrothermal, chemical, enzymatic, and microbial treatment methods have been developed to decompose keratin waste for various industrial applications. Traditional physical and chemical methods for processing them are expensive, energy-intensive, and environmentally unfriendly, and their use can destroy some essential amino acids in proteins (Nad et al., 2024; JL and Dhanasingh, 2025). It is worth noting that a small portion of the feather by-product is processed into feather meal to use as a feed additive or fertilizer. However, feather meal is characterized by low digestibility and insufficient levels of essential amino acids. The use of keratinolytic microorganisms or their keratinases solves this problem by increasing the amino acid content. Furthermore, biodegradation can ensure cost-effectiveness, process safety, and reduced carbon dioxide emissions (Anbesaw, 2022; Nad et al., 2024).

Thus, from an environmental and economic perspective, the biodegradation of keratinous wastes by microorganisms and their keratinolytic enzymes is considered the most effective alternative for their processing and valorization. Therefore, due to the significant advantages over traditional processing methods and the potential to convert keratin waste into value-added products, microbial degradation of keratin waste has been widely studied in recent years.

Keratins are water-insoluble and highly resistant to degradation by animal or plant proteolytic enzymes due to their high cysteine content, intermolecular disulfide-bond cross-linking, hydrogen bonding, and hydrophobicity (Feroz et al., 2020). Based on their sulphur content, keratins can be divided into two groups, including hard keratin (containing 5% sulphur), which is found in feathers, horns, hair, and nails, and soft keratin (containing 1% sulphur), which is found in skin and calluses (Wang et al., 2016; Anbesaw, 2022). Among the keratin proteins, the most common secondary structures are α-keratin and β-keratins. α-Keratin with a molecular weight of 60-80 kDa is the major protein of mammalian fibers, nails, hooves, and horns, and is characterized by an α-helical structure stabilized by hydrogen and disulfide bonds. α-Keratin has a lower sulfur content compared to other keratins. β-Keratins with a molecular mass of 10-22 kDa are found in the feathers, claws, and scales of reptiles and birds.

These keratins have a twisted β-sheet-like structure consisting of β-strands packed laterally, which can have a parallel or antiparallel orientation. The β-sheets are held together by hydrogen bonds and the planar nature of the peptide bond, which results in the stable pleated β-sheet (Martinez et al., 2020). Finally, γ-keratin is a globular protein with a molecular weight of approximately 15 kDa. The high-sulfur keratin, together with associated proteins, forms the matrix between the microfibrils of mammalian fibers and stabilises the structure of the cortex through the formation of extensive disulfide bridges (Feroz et al., 2020; Martinez et al., 2020).

The ability to degrade keratin is widespread among all groups of microorganisms. Keratinases (EC 3.4.21/24/99.11) are unique enzymes that hydrolyse both soft and hard keratin proteins (Lange et al., 2016). Keratinases are serine or metalloproteases that exhibit activity at temperatures of 30-100°C and a wide pH range,

with an optimum in neutral and alkaline environments (Martinez et al., 2020; Qiu et al., 2020). Keratinases have broad substrate specificity, hydrolysing not only keratins but also haemoglobin, fibrin, gelatine, and casein (Martinez et al., 2020; Qiu et al., 2020). Therefore, the use of keratinases to deconstruct keratin materials is a promising method for obtaining animal feed, plant fertilizers, pharmaceuticals, tannery and leather, cosmetics, detergents, bioplastics, glues, and other products (Jagadeesan et al., 2020; Peng et al., 2020). Compared with microbial degradation, enzymatic hydrolysis is faster and can be regulated to produce hydrolysates with desired composition, making it more suitable for industrial applications (Srivastava et al., 2020; Ossai et al., 2022).

Recently, many studies comprehensively reviewed keratin waste management strategies, the physical and chemical properties of different keratin wastes, their bioconversion using keratinolytic bacteria and microscopic fungi, the commercial value of keratinolytic microbes and enzymes for different applications, keratinase assays, and catalytic properties (Martinez et al., 2020; Qiu et al., 2020; Anbesaw, 2022; Nad et al., 2024; JL and Dhanasingh, 2025). However, to the best of the authors' knowledge, there is no review of the physiology of keratin material degradation and keratinase production by Basidiomycota and Ascomycota fungi, as well as the most important aspects of modulating enzyme activity by changing cultivation conditions. In nature, basidiomycetes, which possess unique enzymatic systems and regulatory mechanisms, play a decisive role in the conversion of lignocellulosic biomass (Tišma et al., 2021; Elisashvili et al., 2023). Basidiomycota fungi represent a taxonomically, ecologically, and physiologically extremely diverse group of eukaryotic organisms. These fungi, belonging to the phylum Basidiomycota, represent a taxonomically, ecologically, and physiologically extremely diverse group of eukaryotic organisms.

Recently, extensive research on these fungi has markedly increased, mainly due to their potential use in a variety of biotechnological applications, particularly for the production of food, enzymes, dietary supplements, and pharmaceutical compounds (Hyde et al., 2019; Elisashvili et al., 2020; Tišma et al., 2021; Łysakowska et al., 2023). However, it has been demonstrated that basidiomycetes can also decompose keratin residues, utilizing them as a source of carbon and nitrogen (Al-Musallam et al., 2013; Inácio et al., 2018; Duffeck et al., 2020). This phenomenon is not unexpected, since most of the nitrogen in lignocellulosic materials is in the form of proteins, and basidiomycetes secrete proteases with broad substrate specificity for their metabolism (da Silva et al., 2025). Therefore, studying the keratinolytic potential of basidiomycetes is necessary to enhance understanding of the mechanism of microbial keratin degradation in nature and to create a platform for developing effective technologies for keratin degradation, making them suitable for use in industry and agriculture. Moreover, searching for saprobic Basidiomycota fungi for the production of keratinase may be a reliable way to exclude the isolation of human pathogens, in particular, dermatophytes. Given the growing need to recycle keratin waste and the demand for keratinases, research involving filamentous fungi has attracted increased attention. However, unlike bacteria, the number

of keratinolytic fungi studied, particularly basidiomycetes, is limited to identifying keratinase overproducers. Furthermore, knowledge of the physiological mechanisms regulating the production of keratin-degrading enzymes in filamentous fungi is fragmentary. In this context, this review aims to gather and generalize up-to-date information on the keratinolytic potential of various Ascomycota and Basidiomycota species to improve understanding of the physiological mechanisms regulating keratin degradation and to maximize keratinase production.

Diversity of keratinolytic Ascomycota and Basidiomycota fungi

Among different industrial enzymes, keratinases have garnered exceptional attention due to their unique catalytic activity and diverse industrial and biotechnological applications (Adelere and Lateef, 2016; Avdiyuk and Varvanets, 2019; Anbesaw, 2022; Kumar and Kumar, 2022). A wide range of microorganisms, including bacteria, actinomycetes, yeasts, microscopic fungi, and basidiomycetes, have enzymes to utilize keratins as their sole source of carbon, nitrogen, and energy. Among them, the most studied are keratinolytic bacteria belonging to the genera *Bacillus, Pseudomonas*, and *Streptomyces* (Avdiyuk and Varvanets, 2019; Kumar and Kumar, 2022). The predominant keratinolytic fungi include the genera Chrysosporium, *Aspergillus, Trichophyton, Trichoderma, Fusarium, Penicillium*, and *Acremonium* (Adelere and Lateef, 2016; Anbesaw, 2022). Compared to mycelial fungi, bacteria are considered more promising for industrial production of keratinases due to their rapid growth, ability to achieve maximum enzyme activity in a shorter time, and ease of scaling up the process (Srivastava et al., 2020; JL and Dhanasingh, 2025). Moreover, bacterial keratinases are distinguished by their efficiency, versatility, and stability, especially those from hyperthermophilic keratinolytic bacteria that survive at temperatures exceeding 80°C (JL and Dhanasingh, 2025). However, it can be hoped that the use of the morphological features of filamentous fungi, the ability of fungal hyphae to attach and penetrate keratin substrates (similar to lignocellulosic substrates), may become a promising approach for the biodegradation of keratin waste and the production of keratinase under solid-state fermentation conditions. Hundreds of microbial strains belonging to various taxonomic groups and isolated from different ecological niches were screened to identify new, effective keratinolytic fungi with the necessary physicochemical and catalytic properties suitable for specific industrial applications. The comparison of the keratinolytic activity of Ascomycota and Basidiomycota fungi allows us to draw several important conclusions (Table 1). Firstly, direct comparison and proper comparative evaluation of data on fungal keratinolytic activity and keratinase yield are difficult due to the use of different keratin materials, fermentation methods, and methods for measuring enzyme activity. Secondly, the ability to degrade keratin is widespread among taxonomically diverse groups of Ascomycota and Basidiomycota fungi. Thirdly, there is interspecific and intraspecific diversity in the potential of mycelial fungi to express keratinolytic activities. Fourth, like most microscopic fungi, basidiomycetes can be used

both as sources of keratinase and for the recycling of keratin waste. Fifthly, isolating a promising degrader of keratin material or a keratinase producer from only keratin waste accumulation sites is not a prerequisite. At least this is true for recently screened basidiomycetes (Khardziani et al., 2026), which were isolated from plant residues and are wood-rotting fungi. Microscopic fungus *Penicillium lanoso-coeruleum,* which produces cold-active keratinase with a maximum activity of 41.7 U/mL (tyrosine), was isolated from cold storage (Alhomaidi, 2025).

Analysis of published data reveals that the keratinolytic activity of filamentous fungi varies significantly, with only a few exhibiting exceptional potential for producing keratinases under suitable cultivation conditions (Table 1). During the fermentation of keratinous materials, the loss of substrate mass is probably the most reliable indicator for assessing the keratinolytic activity of fungi. However, the duration of fermentation of keratin materials in experiments by different authors varied from 4 days to several weeks (Table 1). Therefore, to compare the keratinolytic potential of individual fungi, it would be more accurate to calculate the decomposition rates of keratin materials per day. When decomposing chicken feathers, this indicator for basidiomycetes ranges from 40 mg/day for *Coriolopsis byrsina* to 62.1 mg/day for *Coriolopsis* gallica, and for micromycetes, from 42 mg/day for *Chrysosporium queenslandicum* to 106 mg/day for *Aspergillus flavus* (Table 1). It is worth noting that under the same cultivation conditions of fungi, the rate of decomposition depended on the type of keratin material. Thus, *Fusarium oxysporum* decomposed swine hair at the fastest rate - 98.6 mg/day, and chicken feathers - at 69.8 mg/day (Preczeski et al., 2020). Regarding fungal keratinase activity, Table 1 shows the results of the keratinase activity assay using three methods: 1) by increasing absorption A595 due to the release of dye from keratin azure, 2) by the release of μmol tyrosine from keratin, and 3) by accumulation of substances absorbing at 280 nm. When comparing keratinase activity measured using keratin azure, *Fusarium oxysporum* exhibited the highest enzyme yields – 243 U/mL (Preczeski et al., 2020), whereas *Trametes gibbosa* and *Coprinopsis sp.*, which actively decomposed chicken feathers, demonstrated low keratinase azure activity, 31 U/mL (Khardziani et al., 2026) and 32 U/mL (Al-Musallam et al., 2013), respectively.

When comparing the keratinase activity of fungi, measured by tyrosine release, the undisputed leader is *Didymella keratinophila*, which secretes up to 8780 U/mL of keratinase (Al-Bedak et al., 2023). Among other Ascomycota fungi, *Fusarium brachygibbosum, Aspergillus sydowii,* and *Aspergillus stelliformis* were also found to be excellent keratinase producers (Alwakeel et al., 2021). At the same time, extremely low keratinase activity was detected in cultures of *Penicillium lanosocoeruleum* (Alhomaidi, 2025) and, surprisingly, in *Myrothecium verrucaria*, only 98.8 U/mL (Gioppo et al., 2009).

Among basidiomycetes, *C. gallica* and *Trametes gibbosa* also produced relatively high levels of keratinase activity, 1290 and 1494 U/mL, respectively (Khardziani et al., 2026). However, when comparing the total keratinolytic activity of fungi by hydrolysis of keratin materials and accumulation of substances absorbing at 280 nm, promising potential was revealed for several

other Ascomycota and Basidiomycota fungi.
Among basidiomycetes, *Pleurotus pulmonarius* showed particularly high keratinolytic activity (Inácio et al., 2018), higher than the best producer among microscopic fungi – *Chrysosporium tropicum* (Koutb et al., 2023). Interestingly, the crude enzymatic extract (22 U) obtained after solid-state fermentation (SSF) of human hairs by *P. pulmonarius* within 48 h of in vitro reaction hydrolyzed 29.5% of human hairs, 23.5% of chicken feathers, 16% of beef skin, and no degradation was observed in relation to the wool (Inácio et al., 2018). It means that the degradation of keratin substrates by the same enzyme preparation likely depends on both the substrate's chemical composition and structure and the enzyme's specificity for the substrate.

Physiological aspects of keratinase production by mycelial fungi

Developing highly productive fermentation processes with enhanced ability of fungi to degrade keratin materials and produce high yields of keratinase is challenging and requires fundamental physiological studies. Compared to bacterial cultures, the physiology of keratin material degradation and keratinase synthesis has not been sufficiently studied. Nevertheless, the available fragmentary literary data indicate that the degradation of keratin materials and the production of keratinase are influenced by many specific factors, and the impact of these factors varies among different fungal species (Hassan et al., 2020; Anbesaw, 2022). It is clear that optimizing the medium composition, establishing favorable environmental factors, and developing more advanced technological processes are necessary to achieve both maximum enzymatic activity and reduce the cost of the enzyme.

Effect of the cultivation method on fungal keratinolytic activity

Table 1 illustrates that studies on the degradation of keratin materials and the production of keratinase have been conducted using both submerged and solid-state fermentation (SSF) of keratin materials. Both methods of culturing microorganisms have their advantages and disadvantages. SSF technology, which imitates the natural habitat of filamentous fungi, has several advantages over submerged fermentation, such as high volumetric productivity and relatively higher product concentration, simpler downstream processing, less wastewater generation, low energy and water consumption, the need for simple fermentation equipment and therefore low capital investment, and greater cost efficiency (Rodríguez-Couto, 2019; Londoño-Hernandez et al., 2020). SSF is a promising strategy for large-scale production of keratinase and especially for bioremediation of keratin waste landfills. Although the majority of studies were performed in submerged cultivation conditions, there are few reports on keratin materials degradation and keratinase production under SSF (Inácio et al., 2018; Oliveira et al., 2019; Masood et al., 2023). However, it should be noted that limited studies were focused on the comparative assessment of the keratinolytic potential of the same fungi in SSF and submerged fermentation of keratin materials (Gioppo et al., 2009; Mazotto et al., 2013; Bagewadi et al., 2018). Thus, Gioppo et al. (2009) carried out submerged fermentation and SSF of poultry feathers using *M. verrucaria* and revealed that this fungus secretes keratinase

equally well, accumulating 93 U/mL and 98.8 U/mL, respectively. Fermentation of a mixture of chicken feathers and cassava bagasse resulted in an increase in *M. verrucaria* keratinase yield to 168 U/mL and 189 U/mL in submerged and SSF, respectively. However, the growth of *Trichoderma harzianum* on feather meal as a substrate for SSF was accompanied by an increase in the production of keratinases by 1.8-fold compared to that of submerged cultivation (Bagewadi et al., 2018). Moreover, SSF by *T. harzianum* yielded significantly higher concentrations of soluble proteins (4.3-11.2 mg/g) than submerged fermentation (0.6-2.1 mg/mL). Interestingly, during submerged fermentation of chicken feathers, keratinase activity increased over a period of 4 days, followed by a decrease in enzyme activity. Under SSF conditions, maximum keratinase production and complete colonization of the substrate by the fungus were recorded on the 8th day (Bagewadi et al., 2018). Mazotto et al. (2013) compared feather degradation during submerged and SSF using feathers as the only carbon source for the cultivation of *A. niger* strains 3T5B8, 9D40, 9D80, and 11D40. They noted that after seven days of fermentation, the barbules were degraded, while the rachides remained intact. The authors measured the content of soluble protein in the supernatants of fungal cultures, since measuring the protein concentration in the supernatant can serve as an indirect indicator of the degradation of the feather substrate. Among the tested strains, after four days of SSF, the supernatant of *A. niger* 3T5B8 culture contained 9.4 mg/mL of protein, which was 1.9, 3.0, and 1.6 times higher than the protein concentration in the supernatants of *A. niger* 9D40, 9D80, and 11D40, respectively, under the same cultivation conditions.

It is noteworthy that protein concentrations in the extracts obtained after SSF were significantly higher than those obtained after submerged fermentation. The study showed that solid-state fermentation of feather keratin could be used to produce keratinases by *Aspergillus niger* strain 3T5B8, which produced 7 times higher keratinolytic activity in the solid-state fermentation than in submerged fermentation (Mazotto et al., 2013). Overall, choosing between SSF and submerged fermentation requires evaluating various possibilities and production needs. Submerged fermentation provides better process control and scalability, which are crucial for industrial production. SSF is attractive for certain biotechnological processes, particularly for on-site, tailor-made enzyme production. Wood-rotting and litter-decomposing basidiomycetes are particularly suitable for SSF because they are adapted to growth on solid lignocellulosic substrates.

Effect of growth substrate and carbon source on the keratinase production

Careful selection of the carbon source (and potential inducer of keratinase synthesis) in the presence of which the enzyme producer grows is of paramount importance. For keratinase production by keratinolytic fungi, the growth medium usually includes keratin or keratinous materials, which serve as their sole sources of carbon and nitrogen and as an inducer of the synthesis of enzymes that decompose keratin polymer (Adelere and Lateef, 2016; Avdiyuk and Varvanets, 2019). Al-Musallam et al. (2013) evaluated the keratinolytic potential of three strains of the thermophilic mushroom

Coprinopsis sp. in their growth in a mineral-based medium containing 1.5 g chicken feather powder as the sole source of carbon and nitrogen. All strains degraded the feathers, as evidenced by a loss in feather dry weight and the release of soluble protein fractions into culture filtrates, as determined by spectrophotometric absorption at 280 nm. However, strain Kucc595 exhibited the highest feather dry weight loss in the first three weeks (64%), whereas strains Kucc573 and Kucc597 caused 34% and 20.3%, respectively, over the same time period, although the measurement of fungal keratinase activity using keratin azure showed approximately the same enzyme activity - 31.8 KU/ml, 28.6 KU/ml, and 28.6 KU/ml for Kucc 595, Kucc 573, and Kucc 597, respectively (Al-Musallam et al., 2013). It can be assumed that there is no correlation between keratin degradation and fungal keratinase activity. However, to obtain accurate conclusions, a comprehensive approach is required, taking into account, for example, the dynamics of accumulation of keratinase activity during the cultivation period or the dynamics of changes in the pH of the medium that affect the keratinase catalytic activity. Keratin materials differ in structure, compactness, and chemical composition; therefore, some authors have studied their susceptibility to degradation and the effects on keratinase production. For example, chicken, duck, goose, and turkey feathers, goat hair, sheep wool, and buffalo horn were tested to compare their suitability as sole carbon and nitrogen sources for culturing *A. nidulans* K7 and *A. tenuissima* K2 (Saber et al., 2010). Both fungi were able to grow and degrade all materials to varying degrees, using them as growth substrates. The CFs were the most sensitive to degradation by both fungi compared to other keratin sources. However, *A. nidulans* K7 possessed better keratinase production and solubilization percent than *A. tenuissima* K2 on different keratinous wastes, although the data presented show that the degree of solubilization of individual keratin materials by the tested fungi depended on their concentration and origin. Furthermore, by varying the concentration of keratin materials from 0.5 to 2% during the cultivation of both fungi, the researchers found that, except for buffalo horn, concentrations of the remaining materials in the range of 1.5-2% were optimal for maximizing keratinase yield and waste solubilization by both fungi (Saber et al., 2010). Using buffalo horn at a concentration of 1% ensured maximum keratinase activity and keratin solubilization by *A. nidulans* K7 and *A. tenuissima* K2. El-Ghonemi and Ali (2017) studied the effect of chicken feather concentrations (5, 10, 20, 30, 40, and 50 g/L) on keratinase production by *Aspergillus* DHE 7 and found that keratinase production by the fungus increased with increasing feather concentration up to 20 g/L, with a maximum activity of 164 U/mL. Swine hair and chicken feathers were used at a concentration of 1% for submerged fermentation with *Fusarium oxysporum* and *Aspergillus sp.* (Preczeski et al., 2020). *F. oxysporum* demonstrated the maximum production of keratinases on day 6 of hair and feathers fermentation, 243 and 149 U/mL, and the degree of degradation of the substrates was 59.2 and 54%, respectively. *Aspergillus sp.* indicated maximum enzymatic activity on day 6 of fermentation of swine hair and on day 9 of fermentation of chicken feathers, reaching 112.3 and 113.5 U/mL, respectively, and the degree of

degradation was 34.7 and 39.1%, respectively. To study the keratinase production by *Arthroderma multifidum* depending on the keratin substrate, Kumawat et al. (2022) used human hair, goat hair, chicken feathers, and human nail clippings. The highest keratinase activity was detected during submerged fermentation of chicken feathers (56 U/mL), followed by human hair (44 U/mL), animal hair (35 U/mL), and human nail clippings (16 U/mL). Among five substrates (human hair, pig hair, feather meal, chicken feathers, and bovine horn) used at a concentration of 10 g/L in an aqueous solution, feather meal appeared to be the best growth substrate for inducing keratinolytic activity (Lopes et al., 2011). The peak of keratinolytic activity occurred in 48 h of cultivation, and keratinolytic activity in this condition was 30 U/mL. Using a central composite design combined with response surface methodology, the authors established that the optimal conditions for keratinase production were a pH of 7.8 and 20 g/L feather meal (Lopes et al., 2011). Overall, all these studies demonstrate the varying specificities and preferences of individual fungal species and even strains with respect to the degradable substrate. Furthermore, maximum keratinase production requires the selection of a species-specific keratin substrate. Keratinases are generally considered to be inducible enzymes, with keratin acting as the inducer of enzyme synthesis. However, it has been shown that some non-keratin substrates can also induce keratinase production. For example, fungal strains of *Doratomyces microspores* and *Paecilomyces marquandii* were cultivated in a submerged fermentation of soy flour as an enzyme inducer (Gradišar et al., 2005). Keratinolytic activity was evident in the culture liquids of both strains after 40 h and reached a maximum of 49.5 U/mL after 95 h of cultivation for *D. microspores* and 230.6 U/mL after 110 h for *P. marquandii*. Mini et al. (2015) compared rice bran, wheat bran, wheat powder, coconut oil cake, groundnut oil cake, and feather keratin as growth substrates for keratinase production under SSF by *Aspergillus flavus*. Keratin powder from feathers (435 U/g), followed by wheat bran (426 U/g), was found to be the best for the enzyme production, whereas groundnut oil cake and coconut oil cake provided extremely low keratinase activity (33-35 U/g). To understand the physiological peculiarities of keratinase production by individual fungi, several research groups have studied the effects of additional carbon sources in combination with keratin substrates. Gioppo et al. (2009) reported that sugars, such as glucose, maltose, and sucrose, added to the feather meal medium only slightly increased keratinase production by submerged culture of *M. verrucaria*. Under the same cultivation conditions, the addition of starch increased keratinase production from 93 U/mL in the control medium to 135 U/mL in the polysaccharide-containing medium.

Furthermore, the addition of cassava pulp to the medium increased keratinase activity to 168 U/mL. It should be noted that the cultivation of the fungus was accompanied by an increase in the pH of all media, although the addition of glucose caused a significant decrease in pH on the first day of fermentation. A study of the keratinolytic activity of *M. verrucaria* in poultry feather SSF revealed that the addition of glucose to the medium slightly improved keratinase production, while cassava pulp increased enzyme production from 98.8 to 189 U/mL.

Saber et al. (2010) investigated the influence of several mono-, di-, and polysaccharides at a concentration of 1% on the keratinolytic activity of submerged cultures of microscopic fungi. The highest keratinolytic activity and feather solubilization were recorded with starch and maltose. Specifically, *A. tenuissima* K2 keratinase activity increased from 53.4 U/mL in the control medium to 75.4 U/mL in the starch-supplemented medium, although feather solubilization increased only from 30.1% to 31.5%, respectively. The lowest keratinase activity and feather solubilization were revealed in the medium supplemented with carboxymethyl cellulose (CMC) - 50.6 U/mL and 22.3%, respectively. When culturing *A. nidulans* K7, the addition of starch to the medium with 1.5% chicken feathers caused an increase in keratinase activity from 55.8 U/mL to 77.4 U/mL, and feather solubilization from 32.1% to 38.6% (Saber et al., 2010). By contrast, mannose, CMC, and pectin negatively affected keratinase production and feather solubilization.

To improve keratinase production by *Aspergillus sp.* DHE7, different carbon sources were added to the basal medium containing chicken feathers at a concentration of 1% (El-Ghonemy and Ali, 2017). Sucrose proved to be the best carbon source for keratinase production, providing 226 U/mL, followed by arabinose (198 U/mL) and glucose (195 U/mL), while maltose, raffinose, and galactose decreased keratinase production. By varying the sucrose concentration from 0.5% to 3.5%, it was found that the maximum production of *Aspergillus sp.* DHE7 keratinase was achieved by culturing at 0.5% sucrose, whereas higher sucrose concentrations act as a repressor of enzyme production.

To study the effect of carbon sources on the biodegradation of feather keratin by three strains of *Trichophyton ajelloi* and the keratinase activity of fungi, Możejko and Bohacz (2022) added glucose and xylose at a concentration of 1% to the basal medium with 1% feathers. The presence of xylose in the medium inhibited keratinase activity during the first 14 days of fermentation. By the end of the experiment, both xylose and glucose stimulated enzyme production, and keratinase activity in the medium supplemented with glucose or xylose was on average 177% and 115% higher, respectively, compared to the control medium.

In the experiments of Mini et al. (2015), six chemically different carbon supplements (2% w/w) lowered keratinase production in the SSF of poultry feathers with the fungal culture *Aspergillus flavus* from 630 U/g in the control medium to 219 (dextrin) and 348 (starch) U/g. Anbu et al. (2007) and Singh (2010) explain this phenomenon by catabolite repression of keratinase synthesis due to the presence of easily metabolized carbon sources.

In summary, the available literature suggests that the presence of keratinous materials in the medium likely plays an important, but not decisive, role in the expression of keratinase activity. Mycelial fungi are capable of metabolizing any keratinous material to supply the culture with carbon and nitrogen sources. However, the rate and extent of decomposition of keratinous materials depend on their chemical composition and structure. It is important to establish an appropriate combination of a specific fungal strain and keratin substrate for maximum keratinase production. The limited data available on the

role of additional carbon sources in keratin degradation and keratinase production are contradictory. This depends on both the chemical structure and concentration of individual carbohydrates, as well as their varying effects on the processes of keratin breakdown and enzyme synthesis. For example, culturing a fungus in the presence of high concentrations of easily metabolizable sugars can be accompanied by strong acidification of the medium. Under these pH conditions, keratinase production may occur at a high rate, but its catalytic activity will be far from optimal, and keratin degradation will proceed at a relatively low rate. Furthermore, the presence of catabolite repression of keratinase synthesis in fungi cannot be ruled out. Therefore, it is necessary to monitor the keratinolytic activity of the fungus and the sugar concentration from the very beginning of cultivation until its depletion in the nutrient medium. In any case, it is important to find the optimal ratio of keratin substrate concentrations and additional carbon source in the medium, taking into account the physiological characteristics and nutritional needs of each specific fungal strain, in order to maximize keratinase production.

Effect of an additional nitrogen source on the keratinase production

Keratin materials are excellent nitrogen sources for various groups of microorganisms. Nevertheless, many research groups evaluated the effects of additional nitrogen sources on keratin degradation and fungal keratinase activity. The addition of individual nitrogen sources has been shown to modulate keratinase production in some microorganisms. Thus, among organic and inorganic nitrogen sources (peptone, yeast extract, beef extract, sodium nitrate, ammonium chloride, and ammonium sulfate; each at 0.2 %) used for the submerged fermentation of native chicken feathers at a 1% concentration by *Didymella keratinophila* AUMC 15399, sodium nitrate provided the best keratinase activity of 2440 U/mL (Al-Bedak et al., 2023). When testing six inorganic and organic nitrogen sources for the fermentation of feather waste with *P. lanosocoeruleum*, peptone was the best, with an activity of 41.7 U/mL, followed by yeast and beef extracts (Alhomaidi, 2025). When studying the SSF of poultry feathers with *Aspergillus flavus*, both organic and inorganic nitrogen-containing supplements at 2% w/w reduced keratinase production from 602-651 U/g in the control to 76-447 U/g (Mini et al., 2015). It should be noted that the greatest decrease in keratinase activity was caused by peptone (76 U/g), whereas in the presence of casein, the enzymatic activity reached 447 U/g. Gioppo et al. (2009) indicated that supplementation of the feather meal medium with organic or inorganic nitrogen sources (casein, soybean protein, gelatin, alanine, and ammonium nitrate) decreased the production of keratinase by submerged culture of *M. verrucaria* from 93 U/mL to values around 20 U/mL. Interestingly, adding nitrogen sources to the feather-based medium simultaneously with carbon sources also significantly suppressed keratinase production. As in other studies, the authors observed that during submerged fermentation, an increase in keratinolytic activity was accompanied by an increase in the pH of the medium, probably due to the accumulation of ammonium ions, products of keratin deamination. A study of the

keratinolytic activity of *M. verrucaria* in SSF of poultry feathers showed that the addition of a nitrogen source to the medium alone or in combination with a carbon source (cassava cake or glucose) suppressed keratinase production by approximately 2-5 times (Gioppo et al., 2009). Saber et al. (2010) evaluated the effect of nine different nitrogen sources and observed that corn steep liquor used as an additional nitrogen source slightly increased *A. tenuissima* K2 keratinase activity from 75.4 U/mL in the control medium to 79.8 U/mL, but decreased feather solubilization from 33.5% to 31.5%. The lowest keratinase activity and feather solubilization were revealed in the medium supplemented with NH4H2PO4 - 47.4 U/mL and 15.8%, respectively. Other nitrogen sources reduced keratinase activity by 10.6 - 18% compared to the control. When culturing *A. nidulans* K7, the addition of nitrogen-containing compounds at a concentration of 0.5% to a medium with 1.5% chicken feathers had no effect or a depressive effect on keratinase production and feather solubilization rates. The lowest keratinase activity and feather solubilization rates were observed in a medium supplemented with NH4H2PO4 -44.1 U/mL and 13.4%, respectively. In the experiments of El-Ghonemi and Ali (2017), none of the additional nitrogen sources used at a concentration of 0.5% had a beneficial effect on the production of the *Aspergillus sp.* DHE7 enzyme compared to the control medium. Overall, the available data indicate that the effect of an additional nitrogen source on fungal keratinolytic activity depends on both the physiological characteristics of individual strains and the chemical nature and concentration of the nitrogen compound. Physiologically acidic salts, such as ammonium sulfate, can acidify the nutrient medium, whereas the metabolism of potassium nitrate can alkalize the medium. It can be assumed that differences in pH affect both fungal growth and the catalytic activity of keratinase. It can be hypothesized that some, if not all, filamentous fungi possess a mechanism for nitrogen catabolite repression of keratinase synthesis in the presence of sufficiently high concentrations of nitrogen in the medium. However, to confirm this hypothesis, further special experiments are needed, especially with the introduction of an additional nitrogen source into the growing fungal culture that actively synthesizes keratinase.

Effect of physical-chemical factors

Physicochemical factors such as the medium pH, the cultivation temperature, aeration, and agitation may play an important role in the production of keratinases by mycelial fungi. Filamentous fungi are aerobic organisms, so aeration of the fungal cultures by culturing them on a shaker or in a fermenter promotes their growth and production of bioactive compounds. For example, it has been shown that agitation stimulates production of keratinase activity by *Aspergillus sp.* (Kanchana and Mesta, 2013). In experiments with Trichophyton ajelloi, a decrease in proteolytic activity and stimulation of keratinase activity were observed in stirred cultures compared to stationary cultures (Możejko and Bohacz, 2022). It has been reported that most keratinophilic microbes thrive well at neutral and alkaline pH, with a range of 6.0 to 9.0 (Lopes et al., 2011; Hassan et al., 2020). Kumawat et al. (2022) found that the initial pH of the culture medium significantly

affects keratinase production in *A. multifidum.* Fermentation of chicken feathers in media with initial pH values from 4 to 9 indicated that the optimal pH for keratinase production is 8.0, but growing the fungus in a medium with an initial pH of 4.0 reduced the keratinase activity of the culture from 57 U/mL to 16 U/mL. Variations in the initial pH of the medium within the range of 3 to 9 during the fermentation of chicken feathers as a substrate for the growth of *Aspergillus sp.* DHE7 revealed that the optimal pH for enzyme production (163 U/mL) was observed at pH 6.0. Enzyme production significantly decreased at both lower and higher pH values (El-Ghonemy and Ali, 2017). Typically, submerged and SSF of keratin materials is accompanied by alkalization of the nutrient medium due to the release of ammonium ions (Bohacz et al., 2020; Możejko and Bohacz, 2022). Slight alkalinization of the medium probably favors the decomposition of keratinous materials, since most keratinases have an optimum catalytic activity at a pH of about 8. Basidiomycota and most Ascomycota keratinolytic fungi are mesophiles growing at temperatures of 26-30°C and efficiently producing keratinases (Anitha and Palanivelu, 2012; Avdiyuk and Varvanets, 2019; Al-Bedak et al., 2023), although *Microsporum gypseum, Aphanoascus fulvescens,* and some species of *Chrysosporium* are thermotolerant (Garg et al., 1985; Korniłłowicz-Kowalska and Bohacz, 2011; Hassan et al., 2020). Literature data indicate that taxonomically, and probably ecologically and physiologically, different mycelial fungi differ in the optimum temperature for the production of keratinases. Thus, *A. multifidum* produced keratinase when cultured over a wide temperature range, from 20 to 70°C (Kumawat et al., 2022).

The highest keratinase activity was observed at 40°C with an enzyme activity of 58.8 U/mL, while the lowest was at 20°C with an enzyme activity of 35.4 U/mL. When cultured on chicken feather medium, *Chrysosporium tropicum* maximum keratinase enzyme production was recorded at 35°C (Koutb et al., 2023). The optimum temperature for submerged fermentation of chicken feathers at a concentration of 1% with *Didymella keratinophila* AUMC 15399 was 30°C with a maximum keratinase activity of 5380 U/mL (Al-Bedak et al., 2023). During submerged fermentation of chicken feathers, *Aspergillus sp.* DHE7 was cultured at temperatures ranging from 25 to 45°C. Maximum keratinolytic activity was achieved at 25-35°C, with optimal activity at 30°C (176 U/mL) (El-Ghonemy and Ali, 2017).

Cultivation of *Trichophyton ajelloi* strains at 20, 28, and 37°C for biodegradation of chicken feather waste showed that a temperature of 20°C contributed to an increase in the proteolytic activity of the fungus, while keratinase activity was highest in strain III cultured at 28°C and was, on average, 48% higher than in other experimental variants (Możejko and Bohacz, 2022). Among all factors, controlling the pH of the medium during keratinolytic fungi cultivation is considered a serious challenge, as it is not controlled throughout the entire solid-state or submerged fermentation processes in flasks. Different pH ranges may be required for maximum keratin degradation and keratinase production. Consequently, changes in the pH of the medium during microbial growth have varying effects on both processes and also affect the stability of the synthesized enzyme. These aspects should be taken into account when

designing the fermentation process and formulating the medium for keratin degradation or keratinase production.

Conclusion

Keratinases are among the most sought-after enzymes for various agro-industrial applications. To meet the growing demand for these biocatalysts and to develop highly efficient production technologies, research groups are focusing on identifying new keratinase super-producers and optimizing fermentation parameters to realize their biosynthetic potential. The composition of the nutrient medium, especially the carbon and nitrogen sources and their concentrations, as well as fermentation parameters such as medium pH, temperature, and aeration, affect both the degradation of keratin materials and the production of keratinase. Therefore, the most important fundamental and technological task is to identify key physiological factors regulating the expression and secretion of keratinases for each specific fungal strain. Elucidation of the existence of mechanisms of induction and repression of keratinase synthesis by carbon and nitrogen sources, depending on their concentration in the medium, remains a fundamental task. Finally, a promising area for future study may be to use a mixed cultivation strategy of fungi and bacteria with complementary enzyme systems that are responsible for synergistic keratin degradation.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding

The authors are grateful to the Shota Rustaveli National Science Foundation of Georgia for the financial support of the project FR-22-18172.

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Table 1. Comparison of the keratinolytic activity of Basidiomycota and Ascomycota fungi

Fungal species	Growth substrate, cultivation method, and duration	Degree of keratin degradation	Keratinase activity, assay method, enzyme substrate	References
Basidiomycota fungi				
Coprinopsis sp.	1.5 g of chicken feathers, SF[a], 21 days	0.96 g (64%), 45.7 mg/day	32 U/mL, keratin azure, A_{595}, 0.01	Al-Musallam et al., 2013
Coriolopsis byrsina	0.5 g of chicken feathers, SF, 8 days	0.32 g (63%), 40 mg/day	ND[b]	Duffeck et al., 2020
Coriolopsis gallica	3 g of chicken feathers, SF, 14 days	0.87 g (30.5%), 62.1 mg/day	1290 U/mL, chicken feathers (µmol tyrosine); 33 U/mL, chicken feathers, A_{280}, 0.01	Khardziani et al., 2026
Pleurotus pulmonarius	4 g of human hair, SSF[c], 15 days	ND[a]	260 U/mL, hair fibers, A_{280}, 0.01	Inácio et al., 2018
Trametes gibbosa	2 g of chicken feathers, SF, 14 days	0.68 g (35.9%) 52.1 mg/day	31 U/mL, keratin azure A_{595}, 0.01;	Khardziani et al., 2026
			1494 U/mL, chicken feathers (µmol tyrosine); 133 U/mL, chicken feathers, A_{280}, 0.01	
Ascomycota fungi				
Alternaria alternata	5% of chicken feathers, SF,	ND	1680 U/mL, chicken feathers (µmol tyrosine)	Alwakeel et al., 2021

	15 days			
Alternaria tenuissima	1.5 g of poultry feathers, SF, 6 days	ND	53.4 U/mL, chicken feathers, A_{280}, 0.01	Saber et al., 2010
Arthroderma multifidum	2% of chicken feathers, SF, 12 days	ND	56.7 U/mL, chicken feathers, A_{280}, 0.01	Kumawat et al., 2022
Aspergillus clavatus	0.5% of chicken feathers, SF, 7 days	ND	55.6 U/mL, keratin, A_{280}, 0.01	Timorshina et al., 2022
Aspergillus flavus	2 g of chicken feathers, SSF, 10 days	1.06 g (53%), 106 mg/day	ND	Masood et al., 2023
Aspergillus nidulans	1.5 g of poultry feathers, SF, 5 days	ND	55.8 U/mL, chicken feathers, A_{280}, 0.01	Saber et al., 2010
Aspergillus niger	1% of chicken feather meal, SF, 2 days	ND	30 U/mL, Azokeratin, A_{440}, 0.01	Lopes et al., 2011
Aspergillus niger	10 g of chicken feathers, SF, 7 days	ND	21.3 U/mL, keratin, A_{280}, 0.01;	Mazotto et al., 2013
	0.4 g of chicken feathers+40 g of wheat bran, SSF, 7 days	ND	172.7 U/mL, keratin, A_{280}, 0.01	
Aspergillus niger	2 g of chicken feathers, SSF, 10 days	0.74 g (37%), 74 mg/day	ND	Masood et al., 2023
Aspergillus sp.	1 g of chicken feathers, SF, 9 days	0.39 g (39%), 43.3 mg/day;	113.50 U/mL, keratin azure, A_{595}, 0.01;	Preczeski et al., 2020

	1 g of swine hair, SF, 6 days	0.35 g (35%), 58.3 mg/day	112.3 U/mL, keratin azure, A_{595}, 0.01	
Aspergillus stelliformis	5% of chicken feathers, SF, 15 days	ND	3336 U/mL, chicken feathers, (µmol tyrosine)	Alwakeel et al., 2021
Aspergillus sydowii	5% of chicken feathers, SF, 15 days	ND	3523 U/mL, chicken feathers, (µmol tyrosine)	Alwakeel et al., 2021
Chrysosporium queenslandicum	2 g of chicken feathers, SSF, 10 days	0.420 g (21%), 42 mg/day	ND	Masood et al., 2023
Chrysosporium tropicum	chicken feathers, SF, 14 days	ND	232 U/mL, keratin, A_{280}, 0.01	Koutb et al., 2023
Chrysosporium tropicum	human hairs, SF, 14 days	ND	76 U/mL, human hairs, A_{280}, 0.01	Kumar and Kumar, 2022
Didymella keratinophila	1% chicken feathers, SF, 10 days	ND	8780 U/mL, chicken feathers, (µmol tyrosine)	Al-Bedak et al., 2023
Fusarium brachygibbosum	5% chicken feathers, SF, 15 days	ND	3554 U/mL, chicken feathers, (µmol tyrosine)	Alwakeel et al., 2021
Fusarium oxysporum	1 g of chicken feathers, SF, 6 days	0.419 g (41.9%), 69.8 mg/day;	149.0 U/mL, keratin azure, A_{595}, 0.01;	Preczeski et al., 2020
	1 g of swine hair, SF, 6 days	0.592 g (59.2%), 98.6 mg/day	243.3 U/mL, keratin azure, A_{595}, 0.01	
Myrothecium verrucaria	2 g of poultry feathers + 1% cassava bagasse, SSF, 4 days;	ND	189 U/mL, poultry feathers (µmol tyrosine)	Gioppo et al., 2009

	1% of poultry feathers + 1% cassava bagasse, SF, 4 days	ND	168 U/mL, poultry feathers (µmol tyrosine)	
Penicillium lanosocoeruleum	1 g of chicken feathers, SF, 6 days	ND	41.7 U/mL, chicken feathers, (µmol tyrosine)	Alhomaidi, 2025
Pyrenophora dematioidea	5% of chicken feathers, SF, 15 days	ND	580 U/mL, chicken feathers, (µmol tyrosine)	Alwakeel et al., 2021
Trichoderma harzianum	2% of chicken feathers, SSF, 7 days	ND	104 U/mL, chicken feathers, µg tyrosine/min	Ismail et al., 2012

[a] Submerged fermentation, [b] Not determined, [c] Solid-state fermentation