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Molecular mechanisms and enzymatic pathways of keratin degradation by soil fungi *Curvularia* and *Chrysosporium tropicum*

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Abstract
Keratin, a resilient structural protein found in feathers, hair, and nails, poses significant challenges for degradation. Soil fungi such as *Curvularia* and *Chrysosporium tropicum* have evolved sophisticated enzymatic pathways to break down keratin, involving keratinases and disulfide reductases. This study explores the molecular mechanisms and genetic regulation of keratin degradation by these fungi, emphasizing their optimal conditions and environmental influences. This study also explores the biotechnological applications of keratinases in waste management, the leather industry, cosmetics, and medicine. Addressing challenges in enzyme stability and activity, and advancements in genetic engineering, we propose future directions for optimizing keratinase production, ensuring environmental safety and enhancing industrial processes. Understanding these pathways provides insights into sustainable biotechnological solutions.

Keywords: Molecular mechanisms, keratin degradation, enzymatic pathways, soil fungi, *Curvularia*, *Chrysosporium tropicum*, keratinolytic enzymes

Introduction
Keratin is a fibrous structural protein found in hair, feathers, nails, and other epithelial tissues. Its high cysteine content forms disulfide bonds, contributing to its remarkable mechanical strength and resistance to proteolytic degradation (Gupta & Ramnani, 2006) [1]. This resistance poses significant environmental challenges, particularly in the disposal of keratin-rich waste such as poultry feathers and hair from various industries (Brandelli, Daroit, & Riffel, 2010) [2]. Soil fungi, including *Curvularia* and *Chrysosporium tropicum*, have evolved sophisticated mechanisms to degrade keratin, making them crucial players in the natural recycling of organic matter (Kunert, 1992) [3]. These fungi produce keratinases, a group of proteolytic enzymes that break down keratin into simpler peptides and amino acids (Onifade, Al-Sane, Al-Musallam, & Al-Zarban, 1998) [4]. Additionally, disulfide reductases facilitate the reduction of disulfide bonds, enhancing keratin's susceptibility to enzymatic hydrolysis (Zhang, Zhang, & Wu, 2014) [5]. Understanding the molecular mechanisms and enzymatic pathways of keratin degradation by these fungi has significant implications for biotechnology. Keratinases have potential applications in waste management, the leather industry, cosmetics, and medicine, offering sustainable solutions for keratinous waste disposal and valuable bioproducts. This paper aims to elucidate the enzymatic pathways, genetic regulation, and environmental factors influencing keratin degradation by *Curvularia* and *Chrysosporium tropicum*, and explore the biotechnological applications and future directions in this field.

Structural Composition of Keratin: Keratin is a fibrous structural protein found in hair, feathers, and nails. It is rich in cysteine, which forms disulfide bonds, contributing to its mechanical strength and resistance to degradation. The primary structure involves sequences of amino acids forming α-helices and β-sheets, stabilized by these disulfide bonds [6].

Equation: Disulfide Bond Formation.

$$\text{Cysteine}_i + \text{Cysteine}_j \xrightarrow{\text{Oxidation}} \text{Cystine} + 2H^+ + 2e^-$$

Explanation: Keratinases break down the peptide bonds in keratin, converting it into simpler peptides and amino acids. The action of keratinases is often complemented by other enzymes such as disulfide reductases, which cleave the disulfide bonds, making the keratin structure more accessible to proteases.

1. Reactants
- **Keratin:** A structural protein that is rich in disulfide bonds, making it resistant to regular proteolytic enzymes.
- **H_2O (Water):** Water molecules are involved in the hydrolysis process, which is a key part of breaking peptide bonds.

2. Enzyme
Keratinase: A specialized enzyme that catalyzes the hydrolysis of keratin. Keratinases are classified based on their catalytic mechanisms into serine, cysteine, aspartic, and metalloproteases.

3. Reaction Process
The keratinase enzyme acts on the keratin substrate in the presence of water, breaking the peptide bonds within the keratin protein. This reaction converts the complex keratin structure into simpler molecules.

4. Products
- **Amino Acids:** The building blocks of proteins, which are released as a result of the hydrolysis of peptide bonds in keratin.
- **Peptides:** Shorter chains of amino acids that are also released during the breakdown process.

Table 1: Key Enzymes in Keratin Degradation

Enzyme	Function	Source Organism
Keratinase	Hydrolyzes peptide bonds in keratin	*Curvularia*
Disulfide Reductase	Reduces disulfide bonds in keratin	*Chrysosporium tropicum*
Proteinase K	Broad-spectrum protease activity	*Curvularia*
Urease	Breaks down urea, aiding in keratin degradation	*Chrysosporium tropicum*

***Source*:** Conclusion of Bhuyar, P., [7], (Brandelli, A., Daroit,) [8], (Sharma, V., Sharma, A.,) [9]

Keratin degradation by soil fungi such as *Curvularia* and *Chrysosporium tropicum* involves several key enzymes. Keratinases are pivotal as they hydrolyze the peptide bonds in keratin, effectively breaking down this tough protein into simpler peptides and amino acids (Brandelli, Daroit, & Riffel, 2010) [10]. Disulfide reductases play a crucial role by reducing the disulfide bonds within the keratin structure, which significantly enhances its susceptibility to proteolytic attack by keratinases (Sharma, Sharma, & Seth, 2017) [10]. Proteinase K, another enzyme from *Curvularia*, exhibits broad-spectrum protease activity, facilitating the degradation of various protein substrates, including keratin (Bhuyar *et al*., 2018) [6]. Additionally, urease from *Chrysosporium tropicum* aids in keratin degradation by breaking down urea, a common byproduct of protein catabolism, thereby supporting the overall breakdown process (Sharma *et al*., 2017) [10]. These enzymes work synergistically to convert resilient keratin into useful byproducts, highlighting their biotechnological potential in waste management and other applications (Brandelli *et al*., 2010) [2]. Understanding these enzymatic pathways is crucial for developing efficient bioconversion processes, promoting sustainable utilization of keratinous waste.

(2) Role of disulfide bond reduction
The reduction of disulfide bonds by disulfide reductases is a critical step in keratin degradation, as it transforms the rigid keratin structure into a more flexible and less resistant form.

Equation [11]
$R\text{-}S\text{-}S\text{-}R + 2H^+ + 2e^- \rightarrow 2R\text{-}SH$
This reaction releases free thiol groups, facilitating further proteolytic cleavage by keratinases.

Enzymatic pathways in *Curvularia* and *Chrysosporium tropicum*
(3) *Curvularia's* Pathway
Curvularia species produce a diverse array of keratinases that synergistically degrade keratin. These enzymes are predominantly secreted extracellularly, initiating keratin degradation outside the fungal cell. Keratinases from *Curvularia* exhibit high specificity and efficiency in hydrolyzing the peptide bonds within keratin, breaking it down into smaller peptides and amino acids. This extracellular secretion mechanism allows *Curvularia* to act effectively on keratinous substrates found in the environment, facilitating the recycling of organic matter [12]. Recent studies highlight the optimization of keratinase production in *Curvularia* through genetic and environmental manipulations, enhancing enzyme yield and activity [13].

(4) *Chrysosporium tropicum's* Pathway
Chrysosporium tropicum employs both extracellular and intracellular mechanisms for keratin degradation. This fungus produces robust disulfide reductases that first reduce the structural disulfide bonds in keratin, weakening its rigid structure. Once the disulfide bonds are reduced, keratinases further hydrolyze the peptide bonds, breaking down keratin into simpler molecules. This dual mechanism, involving both reduction and hydrolysis, enhances the overall efficiency of keratin degradation. Recent advancements in understanding the enzymatic pathways of *C. tropicum* have revealed significant potential for biotechnological applications, particularly in the management of keratin-rich waste materials [14].

Equation: Overall Keratin Degradation Pathway.

$$\text{Keratin} + H_2O + \text{Disulfide Reductase} \xrightarrow{\text{Keratinase}} \text{Peptides} + \text{Amino Acids} + \text{R-SH}$$

The equation represents the overall keratin degradation pathway. Disulfide reductase reduces disulfide bonds in keratin, making it more susceptible to enzymatic attack. Keratinase then hydrolyzes the peptide bonds in keratin, breaking it down into peptides, amino acids, and R-SH (reduced sulfur groups). This process highlights the

synergistic action of multiple enzymes in breaking down the tough keratin structure.

Genetic regulation of keratinolytic enzymes

(5) Gene Clusters and Regulatory Elements

The genes encoding keratinolytic enzymes are frequently organized in clusters within the fungal genome. These gene clusters encompass not only keratinases but also disulfide reductases and other accessory proteins essential for keratin degradation. This organization facilitates coordinated expression and regulation, ensuring efficient keratin breakdown. Regulatory elements such as promoters, enhancers, and transcription factors play critical roles in modulating gene expression. Promoters initiate transcription, while enhancers boost transcription levels. Transcription factors bind to these regulatory regions, influencing the timing and extent of gene expression. Recent studies highlight the intricate regulatory networks governing keratinase gene clusters, underscoring the importance of precise genetic control in optimizing enzyme production and activity [15].

(6) Induction and Repression Mechanisms

The production of keratinolytic enzymes is finely tuned by environmental cues. Inducers such as keratin fragments or specific nutrients can upregulate the expression of keratinase genes. This induction mechanism ensures that the enzymes are produced only when keratin substrates are available, optimizing energy use by the organism. Conversely, repressors can inhibit enzyme production under unfavorable conditions, conserving resources. The balance between induction and repression mechanisms is crucial for the adaptive response of fungi to varying environmental conditions. Advances in understanding these regulatory mechanisms have led to improved strategies for manipulating keratinase production in industrial applications, enhancing the efficiency of keratinous waste degradation [16].

Environmental factors influencing keratin degradation

Keratin degradation by microorganisms is significantly influenced by various environmental factors, including pH, temperature, and substrate availability. These factors play a crucial role in optimizing the activity and stability of keratinolytic enzymes, thereby enhancing the efficiency of keratin degradation.

(7) pH: The activity of keratinases is highly pH-dependent. Most keratinases exhibit optimal activity in neutral to alkaline pH ranges (7-9). Extreme pH levels can denature the enzymes or alter the ionization states of amino acid residues essential for catalysis, thereby reducing their activity. Recent studies have demonstrated that keratinases from certain fungal species maintain high activity even under slightly acidic conditions, broadening their potential applications (Gurav, R. G., & Jadhav, J. P. (2013) [17].

(8) Temperature: Temperature is another critical factor affecting keratinase activity. Optimal temperatures for keratinase activity typically range from 30°C to 50°C. Higher temperatures can increase the reaction rate up to a certain point but may lead to enzyme denaturation if the temperature exceeds the enzyme's stability threshold. Conversely, lower temperatures can slow down enzymatic reactions, reducing the overall degradation efficiency. Advances in protein engineering have enabled the development of keratinases with enhanced thermal stability, making them suitable for industrial applications.

(9) Substrate Availability: The presence of keratin-rich substrates in the environment is crucial for the induction of keratinolytic activity in microorganisms. Keratinase production is often significantly upregulated when keratinous materials, such as feathers or hair, are present. This adaptive response ensures that enzymes are synthesized only when needed, optimizing energy expenditure by the microorganisms. The production of keratinase typically kick-starts in the presence of keratinous biomass and the absence of easily available nutrients, which drives the microorganisms to utilize the keratin as a nutrient source (Gupta & Ramnani, 2006; Nnolim *et al*., 2020) [18, 19].

Biotechnological applications of keratinases

Keratinases are specialized proteolytic enzymes that degrade keratin, a tough and insoluble protein found in feathers, hair, and nails. These enzymes have gained significant attention for their diverse biotechnological applications due to their ability to convert keratinous waste into valuable products.

1. Waste Management: Keratinases play a crucial role in the eco-friendly management of keratin-rich waste such as poultry feathers, hair, and wool. Traditional disposal methods like incineration and landfilling are environmentally harmful. Keratinases biodegrade keratinous waste into amino acids and peptides, which can be repurposed as animal feed additives or fertilizers. This approach not only mitigates environmental pollution but also recycles waste into useful products (Gupta & Ramnani, 2006; Brandelli *et al*., 2010) [1, 2].

2. Animal Feed: Hydrolyzed keratin products, rich in amino acids, serve as nutritional supplements in animal feed. This recycling method provides a cost-effective protein source for livestock, enhancing their growth and health. Keratinase-treated feather meal has been shown to improve the nutritional quality of feed, supporting better animal development.

3. Leather Industry: Keratinases are used for dehairing animal hides in the leather industry. Traditional chemical dehairing methods can damage hides and produce toxic effluents. Enzymatic dehairing with keratinases is gentler, preserving hide quality and reducing environmental pollution. This application is essential for producing high-quality leather products. (Fang *et al*., 2019; Duffeck *et al*., 2020) [20, 21]

4. Cosmetic Industry: In cosmetics, keratinases are used for skin exfoliation and rejuvenation. These enzymes break down dead skin cells, promoting a smoother and more youthful appearance. Products containing keratinases are also effective in treating calluses and hyperkeratosis.

5. Textile and Detergent Industries: Keratinases are utilized in the textile industry to process wool and silk, improving fabric softness and quality. In detergents, keratinases enhance stain removal, especially for protein-

based stains, by breaking down proteinaceous materials more effectively.

6. Biomedical Applications: Keratinases have potential biomedical applications, including wound healing and drug delivery. Their ability to degrade keratin can be harnessed for treatments of fungal infections and enhanced drug delivery through the skin. Research is ongoing to explore these therapeutic uses.

7. Agricultural Applications: Keratinase-treated waste can be used as a biofertilizer, enriching soil with nitrogen and other essential nutrients for plant growth. This not only recycles organic waste but also reduces reliance on chemical fertilizers, promoting sustainable agricultural practices.

Challenges and future directions

Challenges

1. **Enzyme Stability and Activity:** One of the primary challenges in the industrial application of keratinases is maintaining their stability and activity under varying environmental conditions. Keratinases often face denaturation and reduced efficiency when exposed to extreme pH levels, high temperatures, or the presence of detergents and salts commonly used in industrial processes (Chen *et al*., 2018) [22]. Enhancing the robustness of these enzymes through protein engineering remains a critical area of research.
2. **Cost of Production:** The cost-effective production of keratinases at a commercial scale is another significant hurdle. Current production methods often involve complex fermentation processes that require precise control of environmental conditions, making them expensive. Innovations in fermentation technology and the use of cheaper substrates could help reduce costs (Fang *et al*., 2019).
3. **Purification Processes:** Efficient and economical purification of keratinases from microbial cultures is essential for their application. Traditional purification methods are often labor-intensive and costly, which can limit the scalability of keratinase production. Developing streamlined and cost-effective purification techniques is crucial (Duffeck *et al*., 2020) [19].

Future Directions

1. **Genetic Engineering:** Advances in genetic engineering and synthetic biology offer promising solutions to enhance keratinase production and stability. Techniques such as CRISPR/Cas9 can be used to modify microbial strains for higher yield and improved enzyme characteristics (Fu *et al*., 2021) [23].
2. **Optimizing Fermentation Conditions:** Research is ongoing to optimize fermentation conditions for maximal keratinase production. This includes using statistical models like Response Surface Methodology (RSM) to fine-tune parameters such as pH, temperature, and substrate concentration (Ghosh *et al*., 2009) [24].
3. **Industrial Applications:** Expanding the use of keratinases beyond traditional sectors like waste management and leather processing to emerging fields such as biomedical applications and biofuel production is a promising area of development. For example, keratinases could be employed in drug delivery systems and as biocatalysts in the production of biofuels from keratinous waste.
4. **Sustainability and Environmental Impact:** Developing sustainable methods for keratinase production and application that minimize environmental impact is essential. This includes using renewable resources as substrates and designing processes that reduce waste and energy consumption.

Conclusion

Keratinases offer significant potential for diverse biotechnological applications, including waste management, animal feed, leather processing, cosmetics, textiles, and biomedical fields. Their ability to efficiently degrade keratinous materials into valuable byproducts underscores their industrial relevance. However, challenges such as enzyme stability, production costs, and purification methods need addressing to maximize their utility. Future research should focus on genetic engineering, optimized fermentation conditions, and sustainable production practices to enhance keratinase applications. By overcoming these challenges, keratinases can play a crucial role in advancing sustainable technologies and contributing to environmental conservation.

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