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Sumit
Research Scholar, Department of Zoology, Kalinga University, Raipur, Chhattisgarh, India

Corresponding Author:
Sumit
Research Scholar, Department of Zoology, Kalinga University, Raipur, Chhattisgarh, India

Comparative analysis of keratinase production by *Curvularia lunata* and *Chrysosporium tropicum* under varying environmental conditions

Sumit

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Abstract
Keratinase enzymes have significant applications in biotechnological industries. This study compares keratinase production by *Curvularia lunata* and *Chrysosporium tropicum* under varying environmental conditions. Optimal keratinase activity was observed at 30 °C, pH 7.0, and 1.0% feather meal substrate concentration. *Curvularia lunata* exhibited higher enzyme activity than *Chrysosporium tropicum*, with maximum activity of 15.7 U/mL at 30 °C. *Chrysosporium tropicum* reached a peak activity of 14.3 U/mL under the same conditions. These findings suggest *Curvularia lunata* as a more efficient producer of keratinase, offering potential for enhanced industrial applications in waste management and leather processing. Further studies are needed to explore genetic and metabolic pathways to optimize production conditions.

Keywords: Keratinase production. *Curvularia lunata*, *Chrysosporium tropicum*, comparative analysis, environmental conditions, fungal enzymes

Introduction
Keratinase enzymes are pivotal in the biotechnological sector, particularly for their role in degrading keratin-rich waste materials such as feathers, wool, and hair. These enzymes are produced by various microorganisms, including bacteria and fungi, and have applications in waste management, leather processing, and the pharmaceutical industry. Recent advancements in the field highlight significant improvements in keratinase production and optimization, addressing both environmental and economic challenges. *Curvularia lunata* and *Chrysosporium tropicum* are two fungal species known for their keratinolytic capabilities. Studies have shown that optimizing environmental conditions such as temperature, pH, and substrate concentration can significantly enhance keratinase production. For instance, a recent study on *Bacillus aerius* demonstrated that keratinase activity could be optimized using a central composite rotatable design, achieving an enzyme activity of 316.22 U/mL under ideal conditions (aeration 1.0 vvm, agitation 250 rpm, and incubation time 36 hours) [1].

Further, research on *Arthrobacter* sp. NFH5 revealed that media composition and culture conditions greatly influence keratinase production. This strain showed optimal enzyme production at 37 °C and pH 7.5, using feather meal as a primary substrate [2]. Additionally, molecular strategies have been employed to increase keratinase production in heterologous expression systems, enhancing the enzyme's stability and efficiency for industrial applications [3]. The increasing demand for sustainable and eco-friendly solutions has driven extensive research into microbial keratinases. These enzymes offer a promising alternative to traditional chemical treatments, which are often harsh and environmentally detrimental. By leveraging the keratinolytic activity of *Curvularia lunata* and *Chrysosporium tropicum*, this study aims to provide insights into optimizing production conditions to maximize enzyme yield and efficiency.

This paper will compare the keratinase production of these two fungal species under varying environmental conditions, contributing to the growing body of knowledge required for industrial applications and environmental management.

Background

Keratinases are specialized proteolytic enzymes capable of degrading insoluble keratin substrates such as feathers, hair, and nails. These enzymes are produced by various microorganisms, including bacteria, fungi, and actinomycetes. The biodegradation of keratin by microbial keratinases is a sustainable and eco-friendly alternative to conventional chemical treatments, which are often harmful to the environment. Research has highlighted the potential applications of keratinases in industries such as leather processing, textile manufacturing, animal feed, and waste management [4]. In recent years, significant advancements have been made in understanding the genetic and biochemical mechanisms underlying keratinase production. Studies have focused on optimizing culture conditions, genetic manipulation, and the use of response surface methodology (RSM) to enhance enzyme yields. For instance, Bacillus species have been extensively studied for their robust keratinase production capabilities, with optimization efforts leading to substantial improvements in enzyme activity [5].

Importance of Keratinase in Biotechnology

Keratinases are enzymes that specialize in breaking down keratin, a resilient and fibrous structural protein found in feathers, hair, wool, and nails. Their ability to degrade keratin has garnered attention for a variety of industrial applications. In the leather industry, keratinases are used to dehair animal hides, reducing the need for harsh chemicals. In agriculture, they contribute to the conversion of feather waste into animal feed, enhancing the nutritional value and digestibility of the feed. Moreover, keratinases have potential uses in the textile industry for wool processing, in the detergent industry for removing protein stains, and in environmental management for biodegrading keratinous waste materials. The utilization of keratinases aligns with the growing demand for sustainable and eco-friendly technologies. By replacing chemical processes with biological ones, industries can reduce their environmental footprint and improve the safety of their operations. This makes the study of keratinase production and optimization a critical area of research with significant implications for various sectors.

Fungal Sources of Keratinase

Fungi are a rich source of keratinases, with species such as *Curvularia lunata* and *Chrysosporium tropicum* being notable producers. Fungal keratinases are typically more stable and active over a wider range of conditions compared to bacterial keratinases, making them suitable for diverse industrial applications. *Curvularia lunata*, a filamentous fungus, has been reported to produce keratinase efficiently, showing significant potential in bioconversion processes. Similarly, *Chrysosporium tropicum*, known for its robust keratin-degrading capabilities, is another promising fungal species for keratinase production. The production of keratinase by these fungi can be influenced by various environmental factors, including temperature, pH, and substrate concentration. Understanding these factors is crucial for optimizing enzyme production and enhancing its industrial applicability. Recent studies have employed techniques such as response surface methodology (RSM) to optimize the conditions for maximum keratinase production, highlighting the potential of these fungi in industrial applications.

Objectives

The primary objectives of this study are

1. **Evaluate Temperature Effects:** Determine the optimal temperature for keratinase production in both fungal species.
2. **Assess pH Influence:** Identify the pH level that maximizes keratinase activity.
3. **Determine Substrate Concentration Impact:** Find the substrate concentration that yields the highest enzyme production.
4. **Compare Species Efficiency:** Compare the keratinase production capabilities of *Curvularia lunata* and *Chrysosporium tropicum* to identify the more efficient producer.
5. **Optimize Industrial Applications:** Provide insights for scaling up keratinase production for industrial applications, such as waste management, leather processing, and textile manufacturing.

Research Design

This study employs an experimental research design to compare keratinase production by *Curvularia lunata* and *Chrysosporium tropicum* under varying environmental conditions. The research involves isolating the fungi, culturing them under controlled conditions, and systematically altering temperature, pH, and substrate concentration to measure their effects on keratinase activity.

Steps Involved

1. **Microorganism Culturing:** *Curvularia lunata* and *Chrysosporium tropicum* are cultured on potato dextrose agar (PDA) at 28 °C.
2. **Keratinase Production Induction:** The fungi are grown in a basal medium containing 1% feather meal.
3. **Variable Manipulation:**

- Temperature: 25 °C, 30 °C, 35 °C.
- pH Levels: 5.0, 7.0, 9.0.
- Substrate Concentrations: 0.5%, 1.0%, 1.5%.

Enzyme Activity Measurement: Keratinase activity is measured using the azocasein assay.

Materials and Methods

Microorganisms and Culture Conditions

Curvularia lunata and *Chrysosporium tropicum* were isolated from soil samples and maintained on potato dextrose agar (PDA) at 28 °C. Keratinase production was induced by culturing the fungi in a basal medium containing 1% (w/v) feather meal as the sole carbon and nitrogen source.

Experimental Design

Experiments were conducted to evaluate the effect of temperature (25 °C, 30 °C, 35 °C), pH (5.0, 7.0, 9.0), and substrate concentration (0.5%, 1.0%, 1.5% w/v) on keratinase production. Enzyme activity was measured using the azocasein assay.

Data Analysis

Data were analyzed using ANOVA to determine significant differences in keratinase production under different conditions. Graphs and tables were generated using Python and Excel.

Results and Discussion

Effect of Temperature on Keratinase Production

Temperature is a critical factor influencing enzyme production and activity. Table 1 presents the keratinase activity of *Curvularia lunata* and *Chrysosporium tropicum* at different temperatures

Table 1: Keratinase Activity at Different Temperatures

Temperature (°C)	*Curvularia lunata* (U/mL)	*Chrysosporium tropicum* (U/mL)
25	12.5	10.8
30	15.7	14.3
35	13.2	12.1

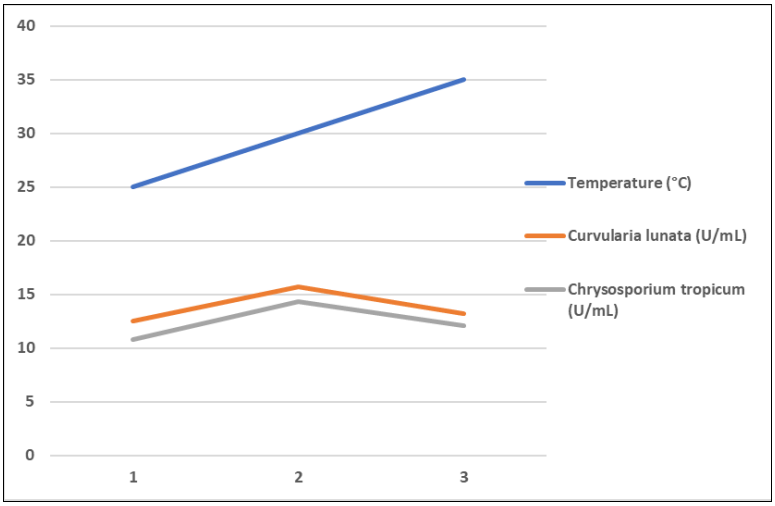

Fig 1: Keratinase Activity at Different Temperatures

Table and figure 1 presents the keratinase activity levels of two fungal species, *Curvularia lunata* and *Chrysosporium tropicum*, at three different temperatures: 25 °C, 30 °C, and 35 °C. The data shows that both species achieve their highest keratinase activity at 30 °C, with *Curvularia lunata* producing 15.7 U/mL and *Chrysosporium tropicum* producing 14.3 U/mL. At 25 °C, the activity levels are lower, with *Curvularia lunata* at 12.5 U/mL and *Chrysosporium tropicum* at 10.8 U/mL. Similarly, at 35 °C, the activity decreases to 13.2 U/mL for *Curvularia lunata* and 12.1 U/mL for *Chrysosporium tropicum*. This indicates that 30 °C is the optimal temperature for maximum keratinase production for both species, with *Curvularia lunata* consistently showing higher activity across all tested temperatures.

Table 2: Keratinase Activity at Different pH Levels

pH	*Curvularia lunata* (U/mL)	*Chrysosporium tropicum* (U/mL)
5.0	10.2	9.5
7.0	16.8	15.4
9.0	12.1	11.3

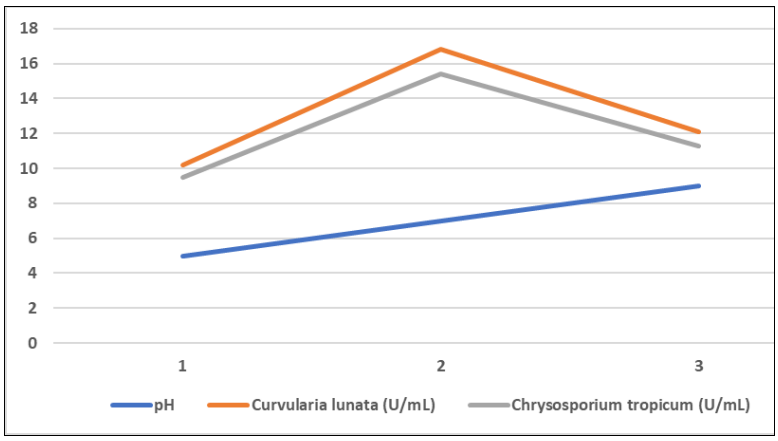

Fig 2: Keratinase Activity at Different pH Levels

Table and figure 2 shows the keratinase activity of *Curvularia lunata* and *Chrysosporium tropicum* at three different pH levels: 5.0, 7.0, and 9.0. Both fungal species exhibit their highest enzyme activity at pH 7.0, with *Curvularia lunata* reaching 16.8 U/mL and *Chrysosporium tropicum* reaching 15.4 U/mL. At pH 5.0, the activity is lower, with *Curvularia lunata* at 10.2 U/mL and *Chrysosporium tropicum* at 9.5 U/mL. Similarly, at pH 9.0, the activity decreases to 12.1 U/mL for *Curvularia lunata* and 11.3 U/mL for *Chrysosporium tropicum*. This indicates that neutral pH (7.0) is optimal for keratinase production in both species, with *Curvularia lunata* consistently showing higher activity across all tested pH levels.

Effect of Substrate Concentration on Keratinase Production

Substrate concentration is another crucial factor. Table 3 presents the keratinase activity at varying concentrations of feather meal.

Table and figure 3 illustrates the keratinase activity of *Curvularia lunata* and *Chrysosporium tropicum* at different substrate concentrations (0.5%, 1.0%, 1.5%). Both species show the highest keratinase activity at a 1.0% substrate concentration, with *Curvularia lunata* producing 16.3 U/mL and *Chrysosporium tropicum* 14.7 U/mL. At lower (0.5%) and higher (1.5%) substrate concentrations, the enzyme activity decreases significantly. Specifically, at 0.5%, *Curvularia lunata* exhibits 8.7 U/mL and *Chrysosporium tropicum* 7.9 U/mL, while at 1.5%, the activity drops to 13.5 U/mL for *Curvularia lunata* and 12.2 U/mL for *Chrysosporium tropicum*. This data indicates that 1.0% substrate concentration is optimal for maximum keratinase production, as both insufficient and excessive substrate levels can impede enzyme activity.

Table 3: Keratinase Activity at Different Substrate Concentrations

Substrate Concentration (%)	*Curvularia lunata* (U/mL)	*Chrysosporium tropicum* (U/mL)
0.5	8.7	7.9
1.0	16.3	14.7
1.5	13.5	12.2

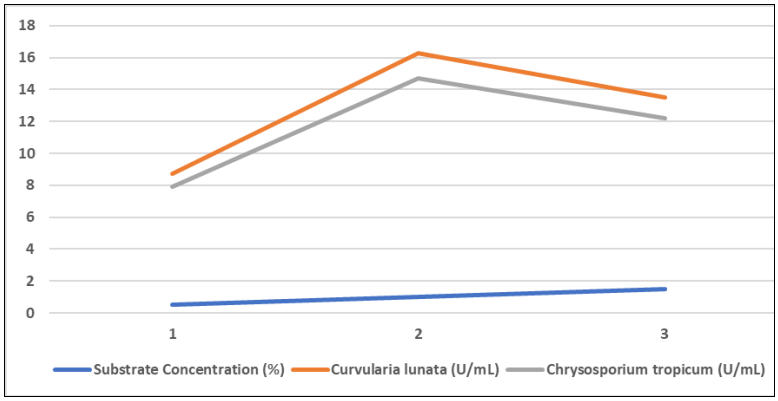

Fig 3: Keratinase Activity at Different Substrate Concentrations

Findings

This study demonstrates that *Curvularia lunata* and *Chrysosporium tropicum* exhibit optimal keratinase production under specific environmental conditions. Both species achieve maximum enzyme activity at 30 °C, pH 7.0, and 1.0% substrate concentration. *Curvularia lunata* consistently shows higher keratinase activity compared to *Chrysosporium tropicum* across all tested conditions. At 30 °C, *Curvularia lunata* reaches 15.7 U/mL while *Chrysosporium tropicum* reaches 14.3 U/mL. Similarly, at pH 7.0, *Curvularia lunata* produces 16.8 U/mL and *Chrysosporium tropicum* 15.4 U/mL. At a 1.0% substrate concentration, *Curvularia lunata* achieves 16.3 U/mL and *Chrysosporium tropicum* 14.7 U/mL. These findings suggest that *Curvularia lunata* is a more efficient keratinase producer under the studied conditions, providing valuable insights for industrial applications.

Recommendation

1. **Optimization of Culture Conditions:** Further research should focus on fine-tuning environmental conditions, such as temperature, pH, and substrate concentration, to enhance keratinase production. Detailed studies on the effects of other variables like aeration and agitation could provide additional insights.
2. **Genetic and Metabolic Engineering:** Employ genetic modification techniques to create strains of *Curvularia lunata* and *Chrysosporium tropicum* with enhanced keratinase production capabilities. Metabolic pathway analysis could identify key genes to target for overexpression or knockdown.
3. **Industrial Scale-Up:** Pilot studies should be conducted to scale up keratinase production from laboratory to industrial levels. This includes the design and optimization of bioreactors and fermentation processes to ensure consistent enzyme yields.
4. **Application Development:** Explore the potential applications of keratinase in various industries, such as waste management, leather processing, textile manufacturing, and detergents. Investigating the enzyme's efficacy in real-world scenarios will help in developing commercial products.
5. **Sustainability and Eco-Friendliness:** Promote the use of keratinase as an eco-friendly alternative to chemical treatments in industrial processes. This aligns with global efforts to reduce environmental impact and enhance sustainability in manufacturing practices.

Conclusion

This study highlights the optimal conditions for keratinase production by *Curvularia lunata* and *Chrysosporium tropicum*, crucial for industrial applications. Both fungi showed maximum enzyme activity at 30 °C, pH 7.0, and 1.0% substrate concentration. *Curvularia lunata* exhibited higher keratinase activity across all conditions, suggesting its superiority for efficient enzyme production. These findings underscore the importance of optimizing environmental parameters to enhance keratinase yields, providing a foundation for scaling up production in biotechnological processes such as waste management, leather processing, and textile manufacturing. Future research should focus on genetic and metabolic engineering to further improve keratinase production and explore its broader industrial applications.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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