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OPTIMIZATION OF ENZYME ACTIVITY AND FEATHER DEGRADATION UNDER VARIED EXPERIMENTAL CONDITIONS BY FUNGI *CURVULARIA LUNATA*

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Abstract

Curvularia lunata, renowned for its distinctive morphological traits and potential for enzymatic activity, serves as a compelling subject for exploring keratinase production and growth optimization. This study delves into the determination of optimal conditions for keratinase production by Curvularia lunata and investigates the correlation between its morphological characteristics and enzymatic activity. Through a series of experiments, the effects of incubation period, temperature, and substrate amount on keratinase production were evaluated. Keratinase activity was measured using a spectrophotometer, revealing significant variations across different conditions. Results indicate that the highest keratinase activity for *Curvularia lunata* was achieved after 16 days of incubation at 25°C, with a substrate amount of 6g. Subsequent purification of the enzyme was conducted via dialysis and column chromatography, resulting in enhanced enzyme activity. The findings underscore the biotechnological potential of Curvularia lunata in keratin degradation processes and contribute to our understanding of optimizing enzyme production for various applications.

Keywords: *Curvularia Lunata*, Keratinase, Enzyme Activity, Growth Optimization, Morphological Characteristics.

1. INTRODUCTION

Keratinophilic fungi are a group of fungi that have the unique ability to degrade keratin, a tough protein found in feathers, hairs, nails, and other epidermal structures of animals.

Macroscopic features of *C. lunata* include brown to black colour, hairy, velvety or woolly texture, and loosely arranged and rapidly growing colonies on potato dextrose agar medium.

Keratinophilic fungi colonize keratinous substrates and convert them to the constituent components of low molecular weight. These fungi can be distinguished from others in their characteristic that they are adapted to consumption of keratinous proteins as sources of carbon and nitrogen. These tend to utilize proteins, peptides, and amino acids as carbon sources even in the presence of sugars (Kumar et al., 2021). These fungi play a crucial role in the decomposition of keratinaceous materials in nature, including feather waste from poultry processing plants.

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Understanding the mechanisms underlying their attachment and degradation of keratin is essential for developing strategies to manage keratin waste, such as feather waste from poultry processing plants, through biological means like fungal biodegradation. Keratin in its native state is not degradable by common proteolytic enzyme such as trypsin, pepsin and papain.

Microbial keratinase is an enzyme that is capable of degrading the insoluble structural protein found in feathers, hair and wool known as keratin. This protein is resistant to degradation by proteolytic enzymes such as trypsin, pepsin, papain due to the composition and molecular conformation of the amino acids bound in the keratin (Meenakshi et al., 2022)

Keratinases are enzymes produced by microorganisms specifically in the presence of keratin-containing substrates. They primarily target the disulfide (-S-S-) bonds present in keratin, which is a structural protein found in feathers, hair, and wool (Bockel et al., 1995).

Research conducted by various scientists has revealed that keratinase production in fungi, Streptomyces, and bacteria occurs predominantly under alkaline pH conditions and at nearly thermophilic temperatures. These enzymes exhibit a wide substrate specificity, capable of degrading not only keratin but also other fibrous proteins like fibrin, elastin, collagen, as well as non-fibrous proteins such as casein, bovine serum albumin, and gelatin (Noval et al., 1959; Mukhapadhayay et al., 1990; Dozie et al., 1994; Lin et al., 1995; Letourneau et al., 1998).

Over the years, numerous studies have focused on isolating and identifying keratinases from different microbial sources. These enzymes have been found in several species of fungi, bacteria, and actinomycetes. Their biochemical diversity and wide-ranging applications make microbial keratinases valuable in various industries, including tanneries, food processing, and waste treatment (Mini et al., 2016).

The unique ability of keratinases to degrade keratin-rich materials has led to their utilization in various biotechnological processes aimed at converting keratin waste into valuable products. Additionally, their application in industries involved in leather processing, food manufacturing, and environmental management highlights the importance of these enzymes in facilitating sustainable and eco-friendly practices.

Curvularia lunata, characterized by its black, downy colonies and distinctive morphological features including erect, unbranched conidiophores and olivaceous brown, smooth-walled conidia, presents an intriguing subject for the study of keratinase enzyme activity and growth optimization.

This research aims to investigate the optimal conditions for keratinase production by Curvularia lunata and explore the correlation between its morphological characteristics and enzymatic activity. By examining the effects of various environmental factors on enzyme production and fungal growth, this study seeks to enhance our understanding of the biotechnological potential of Curvularia lunata in keratin degradation processes (Wang et al., 2022)

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2. METHODOLOGY

For the production of enzymes, pre-cultured and identified fungal material of Curvularia lunata, was utilized. Culture conditions were standardized using Potato Dextrose Agar (PDA) as the culture media, sterilized in an autoclave. The production media composition included yeast extract, di-potassium hydrogen phosphate, potassium dihydrogen orthophosphate, calcium chloride, and magnesium sulphate, with varying concentrations of glucose and peptone as carbon and nitrogen sources, respectively. Additionally, the amount of chicken feathers, serving as the substrate, was varied accordingly. Glasswares utilized were of borosilicate quality, and chemicals such as glycine, sodium hydroxide, and tri-chloro acetic acid were employed in the process. Slants were prepared for long-term preservation of cultures, inoculated with Curvularia species, and incubated under specified conditions. The optimization of enzyme production parameters, including temperature, duration, and substrate amount, was conducted, followed by enzyme assay to assess the efficacy of the process.

2.1. Experimental design

Table 1: Variables and their levels for central composite desigm

	PARAMETERS	-2	-1	0	+1	+2
X1	Duration (days)	4	8	12	16	20
X2	Temperature (°C)	20	25	30	35	40
X3	Feather amount (gms)	3	4	5	6	7
X4	Carbon source (gms)	1	2	3	4	5
X5	Nitrogen source (gms)	4	4.5	5	5.5	6

Table 2: Central composite design arrangement

Serial no.	X1	X2	X3	X4	X5
1	0	0	2	2	-2
2	2	0	2	2	2
3	-1	0	-2	-1	0
4	-1	1	-2	1	0
5	2	0	-1	2	2
6	1	0	0	0	2
7	1	1	-2	2	2
8	2	1	0	2	2
9	0	0	1	-2	-1
10	-2	-2	0	0	0
11	-2	-2	-1	0	0
12	2	-2	-2	2	2
13	-2	-2	2	2	-2
14	-1	-2	2	2	-1
15	-1	-2	1	1	-2
16	2	2	-1	0	-1
17	-2	1	1	-1	-2

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18	-2	0	-2	2	-2
19	-2	1	-1	2	-1
20	-1	2	2	-1	0
21	1	2	0	-2	2
22	0	2	-2	2	1
23	-2	2	1	0	-2
24	-2	2	2	1	0
25	-2	-1	1	2	-2
26	-1	-1	2	1	0
27	0	-1	-2	0	1
28	1	-1	-1	-2	0
29	1	-1	-2	1	2
30	2	-1	0	-1	-1

2.2 Production of Enzyme

The production media, following the method outlined by Ramnani and Gupta (2004), was prepared for each experimental run. In each run, flasks were designated for different conditions: one flask served as a control with media and feathers but no inoculation, while others were inoculated with Curvularia species-containing medium and no feathers, or with both medium and feathers. Inoculation was conducted using an inoculating needle. The flasks were then incubated at varying temperatures ranging from 20°C to 40°C for durations of 4, 8, 12, 16, and 20 days.

2.3 Preparation of Crude Enzyme Extract

Following incubation, the production medium containing the enzyme was filtered through pre-weighed filter paper, and the filterate was collected in small bottles. The filter paper contents were dried in an oven until moisture was completely removed and weighed to estimate the degradation of feathers. Centrifugation was performed at 5000rpm for 5 minutes, and the supernatant was collected as crude enzyme extract, which was then stored at 4°C for further use.

2.4 Enzyme Assay and Parameters Optimization

For enzyme assay and optimization of parameters, test tubes were labeled accordingly for each run. Crude enzyme from the respective flasks was added to the test tubes, along with 20mg of feathers. Control tubes received 3ml of 0.05M Glycine-NaOH buffer (pH 10), while other tubes received 4ml. Incubation was carried out at 60°C for 60 minutes, followed by the addition of 5% TCA to stop the reaction. After incubation at room temperature for 30 minutes, the contents were filtered, and the supernatant was collected for absorbance measurement at 280nm using a UV spectrophotometer.

2.5 Preparation of 0.05M Glycine-NaOH Buffer (pH 10)

A solution of 0.05M Glycine-NaOH buffer (pH 10) was prepared by dissolving 0.375g of Glycine in 100ml of distilled water and 0.2g of NaOH in 100ml of distilled water.

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Subsequently, 30ml of NaOH solution was added to 50ml of Glycine solution to achieve pH 10, and the final volume was adjusted to 200ml with distilled water.

2.6. Preparation of 5% TCA Solution

A 5% solution of tri-chloro acetic acid (TCA) was prepared by adding 5ml of TCA to 95ml of distilled water.

3. RESULTS

3.1. Degradation of feather by the fungi

Table 3: Degradation of feather by the fungi *Curvularia lunata*

	% of feather degradation(by weight)
Run no.	*Curvularia sp.*
1	38.0
2	38.6
3	66.7
4	76.7
5	57.0
6	98.0
7	90.0
8	78.8
9	66.0
10	92.0
11	8.0
12	10.2
13	92.0
14	34.0
15	42.7
16	82.0
17	84.4
18	94.0
19	82.5
20	64.6
21	20.4
22	88.7
23	8.5
24	92.1
25	43.7
26	44.0
27	9.4
28	34.5
29	12.0
30	98.4

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The table displays the percentage of feather degradation by the fungus Curvularia sp. in various experimental runs. Each run represents different conditions. For example, Run No. 6 shows high degradation (98.0%), while Runs No. 11, 23, and 27 exhibit lower rates (8.0%, 8.5%, and 9.4% respectively). These results indicate the variability in Curvularia sp.'s efficiency under different conditions, which is valuable for optimizing feather degradation processes.

3.2. Optimization of Cultural Conditions of Keratinase:

3.2.1 Effect of Temperature on Keratinase Production

Table 4: Effect of temperature on keratinase production

Fungi	TEMPERATURE (°C)				
	20	25	30	35	40
Curvularia sp.	45.9	54.1	98.2	80.6	70.4

The table illustrates the activity of the fungus Curvularia sp. at different temperatures (°C). At 30°C, the highest activity of 98.2% was observed, while activity decreased at both lower and higher temperatures, indicating an optimal temperature range for Curvularia sp. enzymatic activity.

3.2.2. Effect of Incubation Time on Keratinase Production

Table 5: Effect of incubation time on keratinase production

Fungi	Incubation period (Days)				
	4	8	12	16	20
Curvularia sp.	95.8	91.1	70.9	123.1	98.1

The table presents the activity of the fungus Curvularia sp. across different incubation periods (days). The highest activity of 95.8% was recorded at 4 days, followed by 91.1% at 8 days. Activity decreased at 12 days (70.9%) and increased again at 16 days (123.1%), then slightly decreased at 20 days (98.1%). This fluctuation suggests an optimal range for enzymatic activity, with variations based on the duration of the incubation period.

3.2.3. Effect of Amount of Substrate on Keratinase Production

Table 6: Effect of amount of substrate on keratinase production

Fungi	Amount of substrate (gm)				
	3	4	5	6	7
Curvularia sp.	60.0	85.1	97.2	73.7	49.5

The table displays the activity of the fungus Curvularia sp. with varying amounts of substrate (gm). The highest activity of 97.2% was observed at 5 gm of substrate, indicating optimal conditions for enzymatic activity. Activity decreased slightly at both lower (3 gm) and higher (7 gm) substrate amounts, suggesting an ideal range for substrate concentration to maximize Curvularia sp.'s activity.

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3.2.4. Enzyme Activity of Extracted Keratinase on the Substrate

Table 7: Enzyme activity of extracted keratinase on the substrate

Serial no.	X1	X2	X3	X4	X5	% feather degradation	Enzyme activity (purified)
1	0	0	2	2	-2	38	45.8
2	2	0	2	2	2	38.6	106.6
3	-1	0	-2	-1	0	66.7	84.2
4	-1	1	-2	1	0	76.7	84.5
5	2	0	-1	2	2	57	72.5
6	1	0	0	0	2	98	59
7	1	1	-2	2	2	90	91
8	2	1	0	2	2	78.8	151.5
9	0	0	1	-2	-1	66	101.5
10	-2	-2	0	0	0	92	52.2
11	-2	-2	-1	0	0	8	62.9
12	2	-2	-2	2	2	10.2	76.5
13	-2	-2	2	2	-2	92	96.6
14	-1	-2	2	2	-1	34	61.8
15	-1	-2	1	1	-2	42.7	78.7
16	2	2	-1	0	-1	82	148.1
17	-2	1	1	-1	-2	84.4	135.9
18	-2	0	-2	2	-2	94	47.3
19	-2	1	-1	2	-1	82.5	75.2
20	-1	2	2	-1	0	64.6	141.2
21	1	2	0	-2	2	20.4	49.1
22	0	2	-2	2	1	88.7	71.8
23	-2	2	1	0	-2	8.5	77.8
24	-2	2	2	1	0	92.1	53.1
25	-2	-1	1	2	-2	43.7	41.4
26	-1	-1	2	1	0	44	80.4
27	0	-1	-2	0	1	9.4	68.4
28	1	-1	-1	-2	0	34.5	73
29	1	-1	-2	1	2	12	68.7
30	2	-1	0	-1	-1	98.4	37.7

The table provides experimental results showing the percentage of feather degradation and the corresponding enzyme activity after purification by the fungus. Each serial number represents a distinct experimental condition, with variations in factors such as X1 to X5 influencing both feather degradation and enzyme activity. There appears to be a relationship between enzyme activity and feather degradation based on the data provided in the table. In many instances, higher levels of enzyme activity correspond to higher percentages of feather degradation. For example, in serial number 8, where enzyme activity is relatively high at 78.8%, the percentage of feather degradation is also substantial at 78.8%. Similarly, in serial number 16, with enzyme activity at 82.0%, there is a notable percentage of feather degradation at 82.0% as well.

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4. CONCLUSION AND DISCUSSION

In the production of keratinase from feather as substrate, the identified strains *Curvuleria sp. was* used. The production media was inoculated for 4, 8, 12, 16 and 20 days and incubated at 30°C for the optimization of effect of incubation period on keratinase production. The enzyme activity was determined using a spectrophotometer. After production from above days, the enzyme assay showed that the maximum activity was at 16 days i.e., 123.1 U/ml for *Curvuleria sp* which means that maximum enzyme was produced after day 12.

The production media was inoculated and incubated 20, 25, 30, 35.40°C for 12 days for the optimization of effect of temperature on keratinase production. The enzyme activity was determined using a spectrophotometer. After production from above days, the enzyme assay showed that the maximum activity was at 30°C i.e., 98.2 U/ml for *Curvuleria sp*

The production media was inoculated and incubated 30°C for 12 days for the optimization of effect of amount of substrate on keratinase production. The enzyme activity was determined using a spectrophotometer. After production from above days, the enzyme assay showed that the maximum activity was at 5 gm i.e., 97.2 for *Curvuleria sp*.

In the next step, an experimental design was set up which included 30 runs. For each run cultural conditions such as temperature, amount of substrate, amount of carbon source, amount of nitrogen source and number of incubation days were maintained. The enzyme produced was filtered using filter paper and was then purified primarily by dialysis and secondary purification was done by column chromatography through sephadex G-57 gel.

The maximum enzyme activity for *Curvuleria sp.* was recorded in run 8 i.e., 151.5 U/ml, which had the cultural conditions as: temperature 25°C, amount of substrate 6 g, amount of carbon source 5 g, amount of nitrogen source 6 g and 20 days of incubation period. Thus maximum enzyme was produced after day 16.

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