



### RESEARCH ARTICLE

## PRODUCTION AND OPTIMIZATION STUDIES OF THE KERATINASE ENZYME BY *BACILLUS TEQUILENSIS* MBR 25

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#### Abstract

Feather-degrading bacteria are the enzyme keratinase-degraded community of micro-organisms that may degrade feathers. They are omnipresent in the food industry in feather waste. This food sector produces large quantities of feather waste that can hardly be treated using conventional chemical and physical methods. Due to the peculiar biochemical structure of the feather, mediated by beta-keratin and reinforced by several disulfide bonds, the degradation of feather waste is typically the most cost-effective and environmentally friendly process. The present study identified a medium for the development of keratinase by shaking flask culture (*Bacillus tequilensis* MBR 25). Feather (1%) and maximum keratinase efficiency have been determined for the highest keratinase output. Keratinase efficiency improved when all parameters were optimized simultaneously.

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#### Introduction:-

The ongoing global consumption of meat as a by-product of the poultry industry produces a large amount of feather waste per year. More than 5 million tons of feathers are produced annually as a waste product from poultry processing plants. The protein in the plum is extremely high with approximately 90% keratin (1). These molecules are organic, fibrous, recalcitrant, and degrading resistant, with the most common proteolytic enzymes. Indigenous keratin is also extremely inert, usually degradable and long-lasting. Planting and disposal of waste may also be necessary, but care must be taken to avoid groundwater contamination. Modern manufacturing processes, such as chemical treatment and stem pressure cooking, can be converted into animal feed. Feathers may be used as tools for a variety of activities, but there are a large number of unused feathers in the forest. Feathers can be untreated and contain a wide variety of bacteria and toxins, including nitrogen oxides, ammonia, and sulfide hydrogen (2). Food is a waste source because it is recalcitrant. Many researchers are therefore keen to turn feathers into value-added products using economic methods. Microbial products are used in the poultry industry to convert feathers into value-added products, such as biofertilizers and animal feed (3).

Keratinase can therefore be a promising and better way to recycle keratin or keratin waste using specific microorganism enzymes. Possible uses of this microbial keratinase have been reported. Amino acids can also be used as animal nutrition for a wide variety of other applications, such as keratotic protein hydrolysis (4). There are several keratinases made up of hydrated enzyme materials. Due to the involvement in the degradation of the unique insoluble keratin substrate and, in general, protein substrate, bacterial keratinases has potential. Enzymes are used for novel applications such as leather, textiles, and detergents that improve the delivery of medicines and medical applications, cosmetics, degrading prions, pesticides, biodegradable film development, and x-ray films in glues,

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foils, and agro-farm degradation. In a population of microorganisms, such as bacteria, Actinomycetes, saprophytic fungi, or dermatophytes, the production of keratinase enzymes and father waste degradation takes place under different ecological conditions (5). The aim of the study was the production of keratinase by new bacillus SPS (*Bacillus tequilensis* MBR 25), which involved the enzyme-optimization conditions.

## Material And Methods:-

### Preparation of bacterial inoculum

The 50 ml of the nutrient bottle is sealed and sterilized in the autoclave at 15 lbs 121 °C at 20 minutes. The medium was then transferred to *Bacillus tequilensis* MBR 25 after cooling at room temperature in a shaking incubation at 37 °C during the day at 150 rpm (6).

### Production of enzyme keratinase

Basal media used for keratin enzyme production on shaking flasks. Medium compositions: feather powder (10 g), NH<sub>4</sub>Cl (1 g), NaCl (1 g), K<sub>2</sub>HPO<sub>4</sub> (0.6 g), KH<sub>2</sub>PO<sub>4</sub> (0.8 g) and MgCl<sub>2</sub>·6H<sub>2</sub>O (0.48 g). The following were the compositions. At 150 rpm and 37 °C at 72 hours, the revolving shaker was mounted on medium size of 50 mL and cultivated with 2.5 mL of inoculum. After 20 minutes of incubation, the fermented broth was centrifuged at 4 °C at 5000 rpm. A cell-free supernatant is collected and used for the development of keratinase (7).

### Keratinase assay

In the Tris – HCl buffer of 0.05 M (pH 8.0), keratin digestion was used to calculate the activity of the enzyme by 1%. Reaction mixtures contain a 1.75 mL substrate and a 0.25 ml enzyme solution. The mixture was then incubated in the 40 °C bath for 10 and 2,0 ml. Reactions were based on trichloroacetic acid (TCA) 0.4M. The TCA-enzyme solution of 2.0 ml was also achieved without the addition of keratin. The mixture was then centrifuged for 30 minutes at 5000 rpm and absorbed at 280 nm. Increased absorption by one unit (U / mL) is defined as 0.01 at 280 nm (A<sub>280</sub>) as determined by the following equation (8).

$$U = 4 \times n \times A_{280} / (0.01 \times 10)$$

Where n is the dilution factor; 4 is the final reaction volume (ml); 10 is the incubation time (min).

### Optimization studies of keratinase

#### Temperature and pH

The media were induced separately at temperatures between 25 °C and 30 °C (25, 30, 37, 40, 45, 50 °C) and at pH5-8 (5,6, 7, 8) to maintain an adequate enzyme optimization temperature. The pH of 0.1 N HCl or 0.1 N NaOH was used for continuous use. As mentioned above, 72 hours of fermentation have been completed.

#### Incubation period

The effect of the incubation period was investigated by fermentation up to 24, 48, 72, and 96 h for the production of keratinases in individual cases for optimize the medium enzyme production.

#### Speed of agitation

Even at 120, 140, 160, and 180 rpm, the effect of agitation speed for keratinase production was independently tested. The test was completed after 72 h.

## Results and Discussion:-

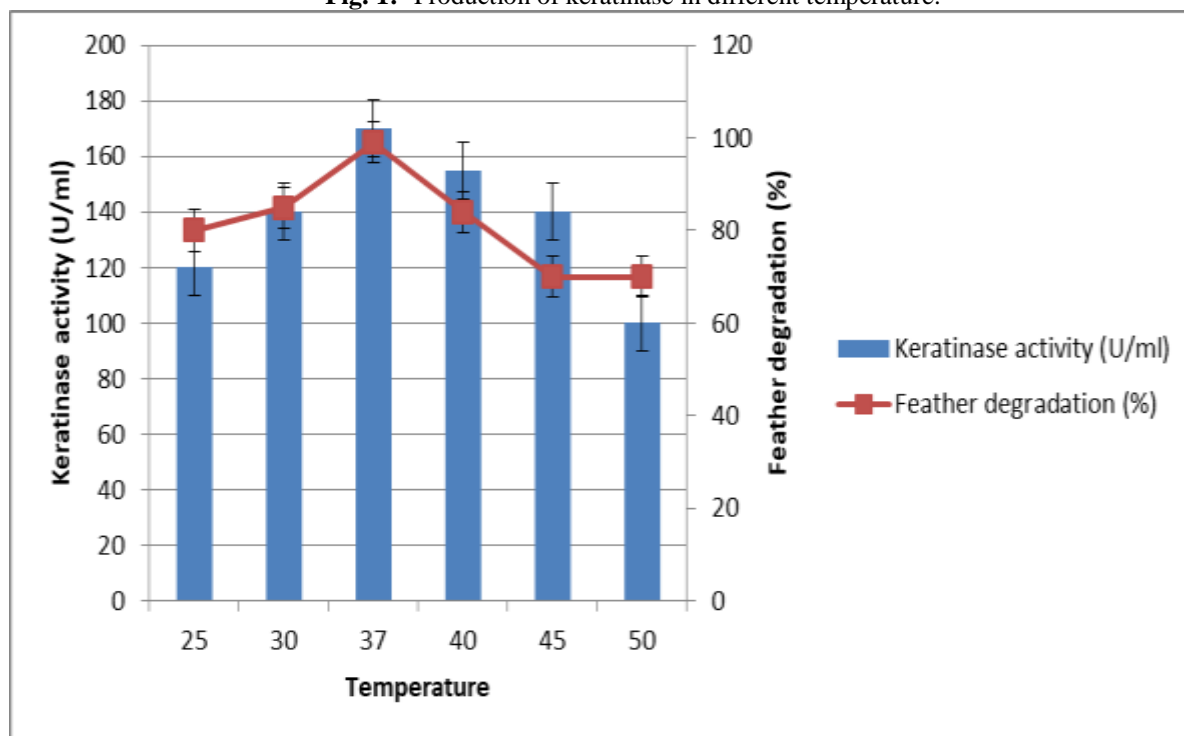
Keratin can form high complexes without chemical modifications, which make it desirable to be used as a new material in many fields. Poultry plumage is a major source of keratin in many fields. It's got great potential (9). Keratins are available from human resources and can be used as medical instruments for their respective products. The quality of keratin derived from human cuttings is used as a new carrier for bone regeneration. Keratins are considered a biodegradable drug used to treat injuries (10). Keratin has become biocompatible and can be used as fresh and useful life-system materials from a range of sources (11).

Amino acids may be converted into food or other waste-keratin, which may be used as animal feed. The feathers of the chickens can be converted from a rat model to a wound healing hydrogel. Feathers consist of oil, water, salt, carbohydrates, copper and nitrogen, potassium and calcium; iron; manganese; copper, and zinc (12). Besides, that also Feather can, of course, be an enormous source of amino acid, and cysteine, glutamine, proline, and serine amino

acids are important in many quantities. Histidine, glycine, tryptophan, and glutamic acid are of low quality (13). Feather hydrolysis may be fed as protein or as an amino feed for animal or microbial development. Feathers may also be used as important sources of carbon and nitrogen for microbial cultivation (14). Soil samples were collected from Hyderabad, Telangana's central poultry waste disposal site. For the selection of possible keratinolytic products in an enrichment system analysis, soil samples were selected. *Bacillus tequilensis* MBR 25 degrades all isolated objects within 48 hours. The accession number MT626712 was obtained from the gene sequence of *Bacillus tequilensis* MBR 25 and was submitted to the NCBI.

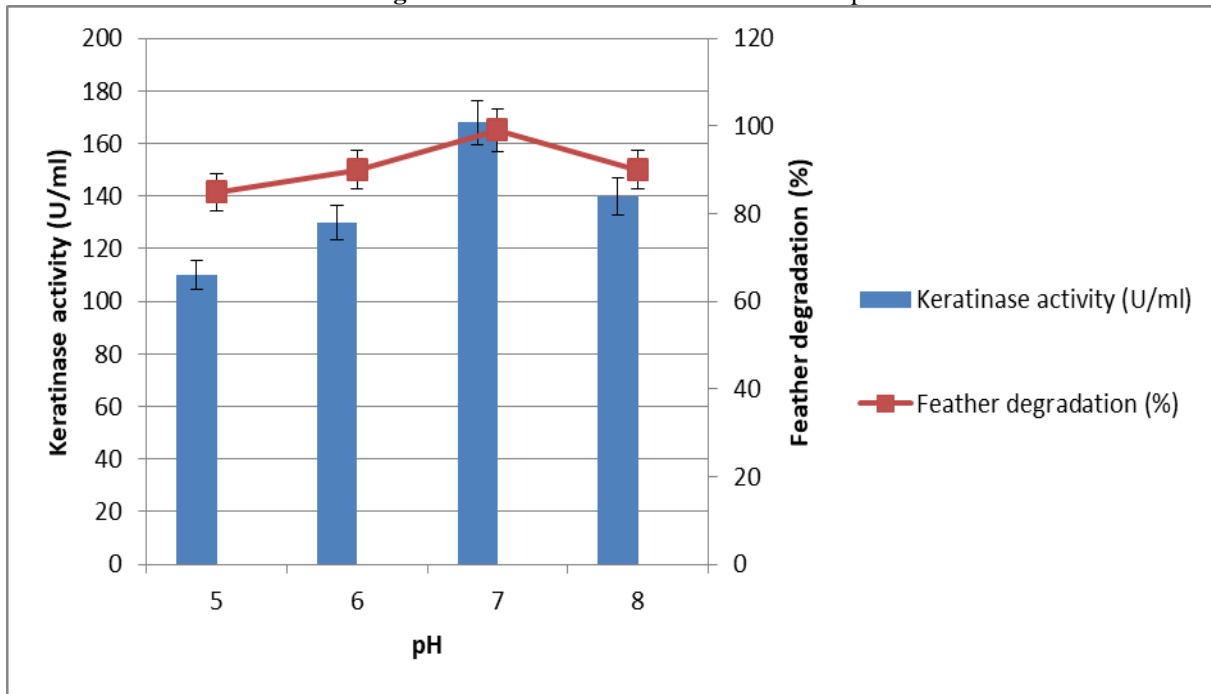
Keratinolytic effects in feathers of all isolates, *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus pumilis* are effectively reduced (15). In the development of keratinolytic proteases containing various proteins, such as casein, feather, and BSA, the enzyme was constitutively produced using the above-mentioned isolates. Microbial keratinase is the inducible enzyme. Isolated *Arthrobacter* sp. NFH5 induced maximum keratinase when keratin protein elements were present in the medium (16). Similar findings were associated with *Arthrobacter*, which recorded the highest keratinase production in the presence of 1% of the feather powder (17). *Bacillus* JB99, keratinase maximum expression in the presence of 1%. Keratinase function depends on the presence and concentration of keratinase. Enzyme development will decrease when the feathers are more frequently concentrated, indicating catabolic suppression (18). In this study, the average enzyme activity selected for *Bacillus tequilensis* MBR 25 in 48 hours was 165 units / mL (reported).

**Fig. 1:-** Production of keratinase in different temperature.



A crucial role in the production of the enzyme is temperature, pH, and other cultural parameters. There is therefore an optimum enzyme production for *Bacillus tequilensis* MBR 25. The production of the enzymes was also high at 35 °C for 24 hours. This is the genus of *Arthrobacter* and other positive keratinase bacteria, such as *Bacillus subtilis* and *Bacillus pumilis* (19), keratinase production temperature was shown to be 40 °C in previous studies. In present study the optimized temperature for keratinase production was 37 °C (Fig. 1).

Fig. 2:- Production of keratinase in different pH.



The pH of the media determines the reaction status of the bacterial cell membrane, enzyme activity, and nutrient up taking. Maximum growth occurs when adequate pH is established in culture media. Optimal pH for the production of *Arthrobacter* sp keratinase is 7.0. In keratinolytic, the pH of the bacteria was stable (20). Further supporters of the present research have concluded that the optimal production of enzymes is moderate pH. In present study the optimized pH for keratinase production was 7 (Fig. 2).

Fig. 3:- Production of keratinase in different Incubation period.

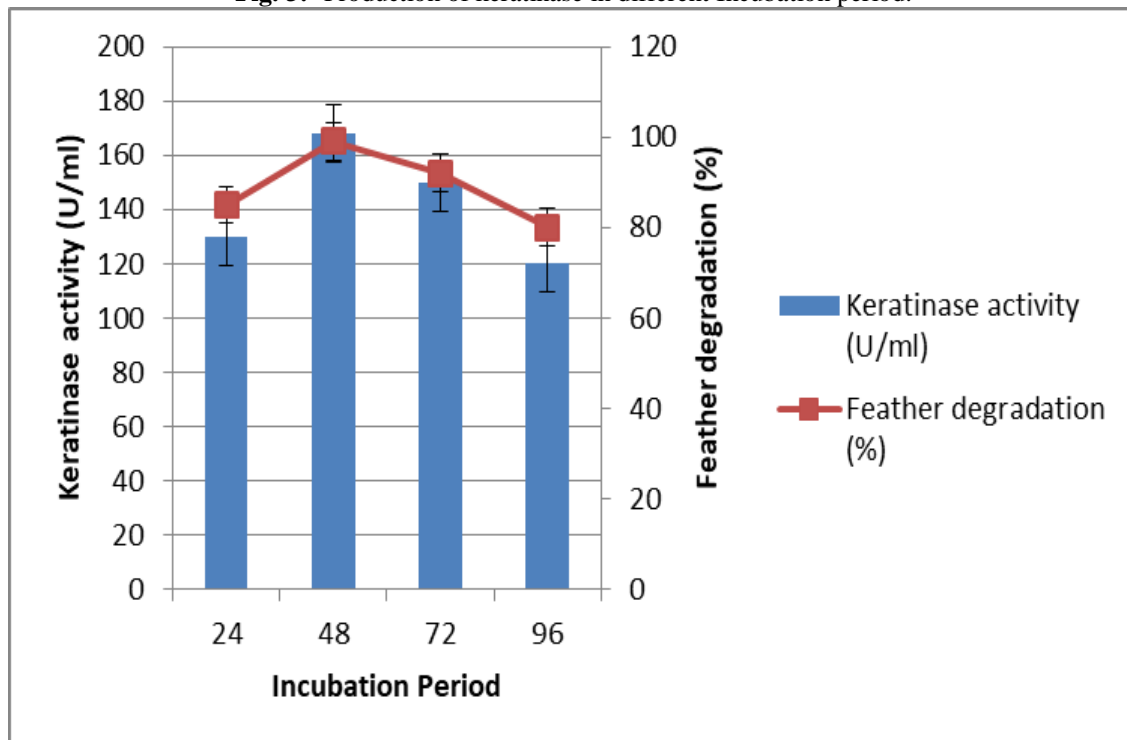
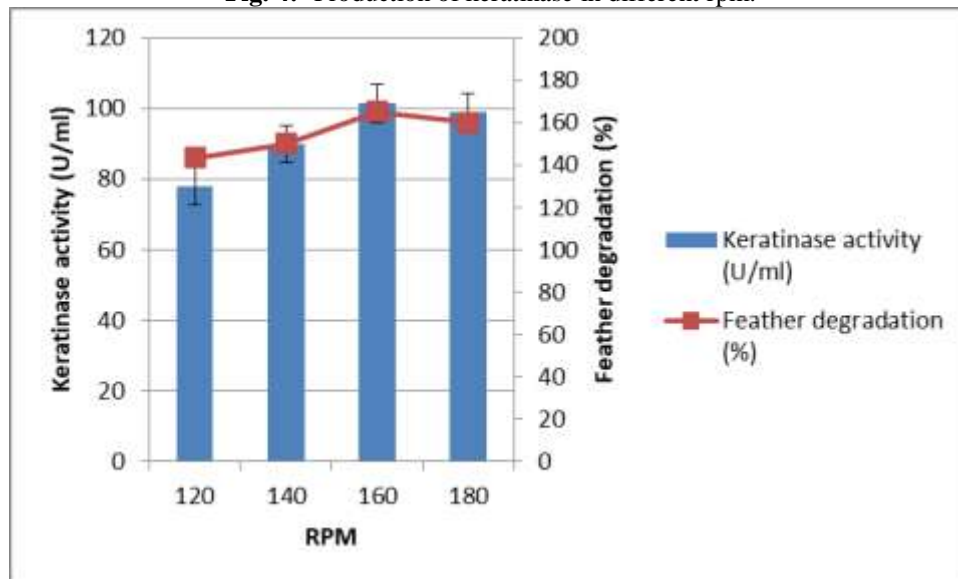


Fig. 4:- Production of keratinase in different rpm.



Several inoculum keratinase concentrations have been measured in the sample. With an increased incubation period, the yield was improved and optimized at 5% (21, 22). In the early stages, outputs will fall sharply with rapid bacterial growth and vital bacterial nutrient depletion. In present study the optimized incubation period for keratinase production was 48 h (Fig. 3).

Table .1:- Final optimized conditions for keratinase production.

Culture conditions		Keratinase activity (U/ml)		Feather degradation (%)	
<b>Temperature</b>					
25		120		80	
30		140		85	
37		170		99	
40		155		84	
45		140		70	
50		100		70	
<b>pH</b>					
5		110		85	
6		130		90	
7		168		99	
8		140		90	
<b>Incubation period</b>					
24		130		85	
48		168		99	
72		150		92	
96		120		80	
<b>RPM</b>					
120		130		86	
140		150		90	
160		169		99	

180		165		96	
Final optimization conditions					
37 °C, pH 7.0, Incubation period 48h and rpm 160		180		100	

#### Data represent the means of triplicate

Higher agitation (200-250 rpm) due to high dissolving oxygen and possibly lower keratin activity has resulted in good bacterial output (23). Substrates and bacteria have been poorly mixed, resulting in the heterogeneous formation and slow dissolution of low turbulence oxygen (24, 25). In present study the optimized rpm for keratinase production was 160 (Fig. 4). The final conditions for high keratinase production along with degradation capacity of feather were optimized (Table. 1) .

#### Conclusion:-

Keratin is a diverse and structurally stable protein, like wings, wings, sticks, and nails, found in hard human and animal tissues. Any such waste, such as fur and fetus, is one of the main sources of high-quality chemicals, such as food, fertilizer, or bio-energy, rich in protein. Recalcitrant and hydrolysis resistance of typical proteases are a major obstacle to the recovery of keratin substrates. However, these substrates can be degraded effectively by special keratinolytic enzymes produced by specific microorganisms. Keratin has also been identified in the pharmaceutical, textile, and leather industries. Increased use of poultry waste, however, often means maximizing the production and use of usable enzymes in other procedures, such as economic pretreatment.

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