

Keratinolytic Activity of *Bacillus* spp. Isolated from Poultry Waste

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ABSTRACT

*Keratinases are the enzymes which hydrolyze keratin. Feathers contain β -keratin which comprises over 90% of feather protein. β -keratin is fibrous and insoluble structural protein extensively cross linked by disulfide bonds. Keratin is a rich source of cystine, and thiosulphate. Bacterial keratinase could be used to degrade feather waste and the digested product could be an excellent material for producing animal feed fertilizer. The present study screened for the hydrolysis of feather keratin by *Bacillus* spp. isolated from poultry farms.*

*Fifteen keratinolytic Bacterial spp. were isolated from poultry farm located at Vita region in Sangli Dist. in Maharashtra. Among them five isolates showed maximum keratinolytic activity towards chicken feathers as a substrate. The degradation of chicken feathers was assessed by determining protein released ($\mu\text{g/ml}$), and weight loss (%). The maximum protein released into the medium from feather degradation under shaking condition was 94.1 $\mu\text{g/ml}$ in 18 days. and maximum weight loss of feathers under shaking condition was 60%. The feathers were turned into brownish powder like mass in 18 days. It was found that complete degradation of feathers by *Bacillus* spp requires eighteen days. under shaking condition. The enzyme activity of keratinase was studied by assay and found to be 276U/ml. In this present study we report the potent keratinolytic *Bacillus* spp which showed 60% degradation of chicken feathers.*

Keywords: Bacterial spp., keratinolytic activity, Feathers, Poultry farms.

INTRODUCTION

Keratin It is the most abundant proteins in epithelial cells of vertebrates and represent the major constituents of skin and its appendages such as nail, hair, feather, and wool.¹ (Bockeal. Feathers are generated in large quantities as a by product of poultry industry.. Now a days feather waste is utilized as a dietary supplement for animal feed stuffs. World-wide poultry processing plants produce millions of tons of feathers as a waste product². Keratinous material is water insoluble and extremely resistant to degradation by common proteolytic enzymes such as trypsin, papain and pepsin. Keratin is highly insoluble protein having fibrous helical structure and disulfide linkages which make it resistant to proteolysis³. The keratin chain is tightly packed in α helix or β sheet into a supercoiled polypeptide chain⁴ resulting in the mechanical stability and resistance for degradation.. Application of appropriate pretreatment methods hydrolyse feather and breaks down its tough structure to small peptides⁵. These methods can lead to destruction of amino acids and decrease protein quality and digestibility. The utilization of agro industrial residues may increase energy conservation and recycling. The use of microbial Keratinase for degradation of keratin and to overcome the loss of amino acids is significant⁶. Keratin belong to class hydrolase and are metalloproteins and efficient proteolytic enzyme⁷. It has been shown that many microorganism such as *Bacillus* spp. *Bcillus licheniformis*⁸, fungi and actinomycetes have ability to degrade keratin from chicken feathers^{9,10}. The use of microbial keratinase in leather and tanning industry would eliminate the utilization of toxic chemicals¹¹.

Keratinase can also be effectively used for bioconversion of poultry waste into feed supplements. Microbial degradation of feathers is beneficial as it improve nutritive value of poultry feathers and reduce loss of energy.

In the present study we report the isolation of keratinolytic bacteria from poultry farms which degrade the feathers effectively within 18days with 60-% degradation.

MATERIALS AND METHODS

Keratinolytic activity of *Bacillus sp.* was studied using chicken feathers.

Poultry soil samples were collected from poultry farms in Vita region in sterile bags and processed on the same day. Samples were diluted by serial dilution method and then streaked on Nutrient agar and Hichrom agar medium¹². The isolates were screened for protease activity by using skim milk agar. The isolate showing large zone of hydrolysis was selected for studying keratinolytic activity.

Chicken feathers were used as a substrate for degradation and to assess the enzyme activity.

A dense suspension of spp. was prepared from three days old culture plates in sterile distilled water. The flasks were inoculated with 1 ml of suspension each. A substrate control was prepared containing 100 ml medium and 200 mg of chicken feathers . The Bacillus control was prepared by adding 100 ml medium and 1 ml o *Bacillus inoculam* in the flask. The test was prepared by adding 100 ml of medium , 200 mg of chicken feathers and 1 ml of *Bacillus inoculam* in the flask. Flasks were incubated at 30 °C under shaking conditions. The degradation of chicken feathers was assessed by measuring weight loss and protein released (mg/ml)¹³.

The degradation of chicken feathers was assessed at different incubation period, i.e. 6,8,10,12,14,16,18,20 and 22 days in order to optimize incubation time. Dry weight of chicken feathers at the end of incubation period was compared to its initial weight in the medium . The removal of degraded feathers were carried out by filtration. The filtrate was centrifuged at 4000 rpm for 10 minutes and the supernatant was assayed for protein determination by Lowry method¹⁴ using Bovine serum albumin as standard. The result of protein estimation was expressed in mg/ml. Keratinolytic activity of the isolate was determined by Keratinase enzyme assay.

RESULTS AND DISCUSSION

Table 1 –weight loss of chicken feathers by *Bacillus spp.*

Days	% weight loss	Protein release under shaking condition($\mu\text{g/ml}$)	Enzyme activity (U/ml)
6	12%	23.0	120
8	20%	35.1	145
10	24%	50.2	150
12	36%	65.5	200
14	44%	70,6	230
16	56%	85.2	250
18	60%	94.1	276
20	52%	75.8	130
22	40%	60.5	112

Graph 1: Enzyme activity of the isolate

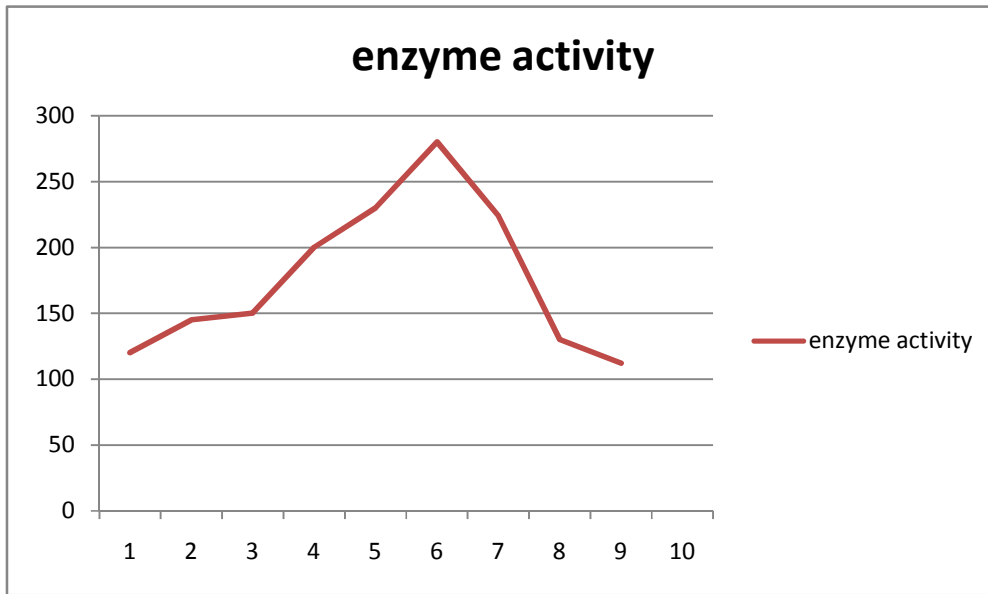


Fig. 1: Growth of the Keratinolytic Bacillus spp. On Hichrome Agar Medium

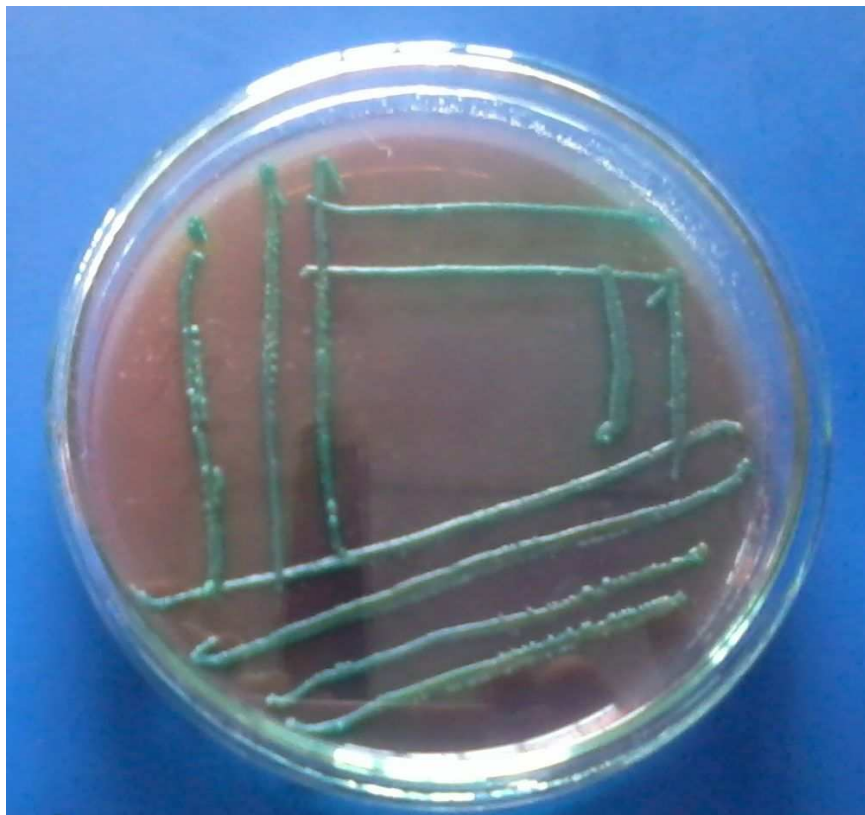


Fig. 2: Protease activity of the isolates on skim milk agar



Fig. 3: Feather degradation of the chicken feathers by the *Bacillus* spp.



The isolated bacterium showed effective degradation of feathers after 18 days. Table 1 represent the weight loss, protein release ($\mu\text{g/ml}$), and enzyme activity during degradation of chicken feathers by *Bacillus* spp. in shaking condition. The degradation of chicken feathers was measured by determining net protein released into the medium and loss of weight at different incubation period. Under shaking condition the maximum net protein release into the medium after degradation was $94.0\mu\text{g/ml}$ in 18 days. The protein level was found to be increasing order after 8 days. i.e. 35.5, 50.2, 65.5, 70.6, 85.2 and $94.1\mu\text{g/ml}$ in 8, 10, 12, 14, 16, and 18 days respectively. It was found decreased after 18 days and was recorded 75.8 and $60.5\mu\text{g/ml}$ in 20 and 22 days respectively. In same conditions, weight loss of chicken feather was recorded -12%, 20%, 24%, 36%, 44%, 56%, 60%, 52% and 40% at same time interval. The maximum weight loss was -60% in 18 days. The chicken feathers were turned into white powdery mass after degradation.

It is known that most keratinases are inducible and very specific towards their substrates like hair, wool, nail and feathers etc¹⁵. Keratinase enzyme is found to be stable at 30°C temp and active. The enzyme activity of the *Bacillus* culture was measured at the same time. The isolated *Bacillus* spp shows high keratinase activity. After 18 days of culturing in Basal salt medium with feathers as sole carbon, nitrogen and energy source, it was found that it could completely degrade feathers. Keratinase activity reached upto 276 U/ml at 30 °C with an initial pH level of 7.0.

The use of microbial strain for Keratinase production and degradation of feathers is very significant. The most studied keratinolytic bacterium is *Bacillus licheniformis* which possess high keratinolytic activity. The isolated strain of bacterium could be used for Keratinase production and to improve nutritive quality of animal feed generated from poultry waste¹⁶.

CONCLUSION

- Keratinolytic microorganism isolated in this work presents high keratinolytic activity
- The isolate could be used for biotechnological application in keratin hydrolysis and recycling of poultry waste for environmental protection.

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