



Keratin Degradation and Enzyme Producing Ability of *Aspergillus Flavus* and *Fusarium Solani* From Soil

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ABSTRACT

Feather keratin is highly resistant to degradation, but some keratinase producing microorganisms can easily degrade these insoluble keratins. These keratinase producing species have an important application in removal of poultry waste and recycled into valuable by product. *Aspergillus flavus* and *Fusarium solani* was isolated from soil using wet mount technique and tested for degradation of keratin production of protease and lipase. Maximum percent degradation was noticed in *F. solani* of 33.6 ± 1.1 in 20 days at $28 \pm 2^\circ\text{C}$. The production of different enzymes such as protease and lipase from *A. flavus* and *F. solani*. Maximum production of protease and lipase enzyme, were noted as *A. flavus* compared the *F. solani*. Protease and lipase enzyme have pH 7.6 and temperature 40°C , 55°C respectively. In the present study, sucrose content (70.6, 61.3), Ammonium phosphate (73.3), Yeast extract (68.6) was noted in both protease and lipase enzyme.

Key Words: Keratinase, Keratin Degradation *Aspergillus flavus*, *Fusarium solani*.

INTRODUCTION

Keratin is an insoluble protein and has a stable structure. The mechanical stability of keratin and its resistance to biochemical degradation depend on tight packing of the protein chains in α -helix (α -keratin) or β -sheet (β -keratin) structures and linkage of these structures by disulfide bonds. In acknowledgement of this distinction, fungi merely inhabiting keratinaceous substrates but lacking manifest keratinolytic activity have sometimes been termed "keratinophilic"[1].

Keratin are divided into two types. α -Keratin: It present in wool, hair, and horn. It is in the form of folded chain. β -Keratin: It present in feather in the form of polypeptide chain.

A fungi grown on keratinous substances which is important natural material occurring in nature mainly in the form of hairs, wools, feathers, horns, hooves, nails, skin and other cornified appendages, constituting natural baits for these fungi [2]. Keratinophilic fungi represent an important component of soil microflora where, they decompose the highly resistant keratin, a proteinaceous substrate. This unique group may exist and proliferate activity in soil under favourable condition, particularly in places, where they can utilize various forms of keratin. Enzymes are complex proteins that cause a specific chemical change in other substances, without being changed themselves. Enzymes are very specific. Each enzyme is designed to initiate a specific response with a specific result. Keratins as well as other protein is generally not recognized as a substrate for common protease. Lipases are hydrolytic enzymes that act in aqueous-organic interfaces, catalyzing the cleavage of ester bonds in triglycerides and producing glycerol and free fatty

acids. However, in environments with low water availability, lipases are able to catalyse esterification, interesterification and transesterification reactions, being thus very versatile biocatalysts [3],[4].

Chicken feathers are composed over 90% protein and produced in large amount as a waste by poultry processing world wide. Accumulation of feathers will leads to environmental pollution and feather protein wastage [5],[6]. Keratin utilization has been reported in variety of organisms including non – filamentous and filamentous bacteria, water moulds and fungi [7]. The present study was aimed at degradation of keratin from basal salt solution. Production of protease and lipase enzyme by proteolytic, and lipolytic fugal species. Analysis of physico- chemical properties and assay of protease and lipase enzyme.

EXPERIMENTAL SECTION

Keratin degradation ability of feather dumped soil was collected from Nagercoil at Kanyakumari District. The samples were collected in sterile polythene bags and brought to the laboratory for further evaluation.

Fungi isolated from soils such as *Aspergillus flavus* and *Fusarium solani* were tested for keratin degradation capability using hen feather and keratin substrates. Substrates were cleaned, washed with dextran, air dried, cut into small fragments, further washed five times with distilled water and air dried. These shorts fragments were further dried at 80°c till constant weight. To support initial growth of fungus was a basal salt solution was employed, which contained (gl⁻¹) K₂HPO₄ 0.4; MgSO₄.7H₂O 0.05; NaCl 0.01; FeCl₃ 0.01 and the p^H was adjusted to 7. To 100 ml Erlenmeyer flask 75 ml of basal salt medium was taken, 1 g keratin substract (hen feather) was added and sterilized at 1.1 kg pressure (cm⁻²)⁻¹ for 15 min. After cooling at 28 ± 2°C for 20 days. A set of control flasks without inoculams was also kept [8].

Production of protease[9],[10].

$$\text{REA} = \frac{\text{Diameter of zone of enzyme activity in mm}}{\text{Diameter of the colony in mm}}$$

Production of Lipase [11],[12],[13].

$$\text{REA} = \frac{\text{Diameter of zone of enzyme activity in mm}}{\text{Diameter of the colony in mm}}$$

Effect of p^H & Temperature on protease & lipase production:

The effect of p^H on activity of protease and lipase produced by *A.flavus* & *F.solani* was carried out using different p^H ranges like 3,4,5,6,7,8,9&10. Adjustments of the p^H were done by addition of hydrochloric acid and 0.1 N sodium hydroxide to achieve activity and alkalinity respectively. The optimization media with the test sample and the protease & lipase assay was done after 24 hrs. The best p^H was concluded by reading the absorbance at 540 nm. The effect of temperature on activity of protease and lipase produced by *A.flavus* & *F.solani* was studied by taking various temperatures ranges like 30,40,50,60,70,80,90&100° C. The optimization media was inoculated with the test samples at different temperatures and the protease and lipase assay was done after 24 hrs.

Protease and Lipase using from different carbon supplementation such as starch, maltose and sucrose. Effect of inoculums on protease and lipase production was studied by incubating the medium with inoculums ranging from 1 -5 %. Various nitrogen sources used for the optimization studies such as ammonium chloride, ammonium phosphate, sodium nitrate, urea, yeast extract, peptone. The culture were added and incubated for 48 hrs. The culture was centrifuged at 10,000 rpm for 10 min at 4° C to separate the clear supernatant. The supernatant was analyzed for protease and lipase activity by casein assay. Estimation of total proteins was done [14].

RESULTS AND DISCUSSION

Maximum keratin degradation was noted in *F.solani* 33.6%. Lowest level of keratin degradation was noted in *A.flavus* 19.2% (Table 1). *A.flavus* & *F.solani* were found positive for protease activity. *A.flavus* & *F.solani* showed maximum protease activity with REA 0.28, 0.04 & 0.14, 0.18 IU/ L respectively (Table

1). The protease and lipase productivity were estimated in the isolated two fungal strains such as *A.flavus* & *F.solani*. The results were presented. (Table-2). The pH optimum (7&6) was suitable for protease and lipase production of *Aspergillus* and *Fusarium* using hen feather as substrate. The temperatures optimum (40&50°C) was suitable for proteases production of *Aspergillus* and *Fusarium* using hen feather as substrate. The protease and lipase activity was optimized using different carbon supplementation medium such as starch, maltose, sucrose (Table – 3). The protease productivity was optimized using different nitrogen supplementation medium such as ammonium chloride, ammonium phosphate, sodium nitrate. The lipase productivity was optimized using different nitrogen sources urea, yeast extract, peptone (Table - 4).

Table 1: Protease and lipase activity by keratin degradation

S. No	Organisms	Weight less of hen feather (%)	Lipase activity			Protease activity		
			Colony (mm)	Activity zone (mm)	REA	Colony (mm)	Activity zone (mm)	REA
1	<i>A.flavus</i>	19.2 ± 1.0	35	5	0.18	28	8	0.28
2	<i>F.solani</i>	33.6 ± 1.1	55	10	0.14	50	2	0.14

Values are expressed as Mean ± Standard Deviation.

Table 2: Assay of protease and lipase production

S.No	Organisms	Protease productivity (IU/ml)	Lipase productivity (IU/ml)
1	<i>A.flavus</i>	0.91 ± 0.007	0.71 ± 0.01
2	<i>F.solani</i>	0.63 ± 0.01	0.83 ± 0.007

Values are expressed as Mean ± Standard Deviation.

Table 3: Carbon sources of protease and lipase

S.No	Carbon sources	Lipase (IU/ml)	Protease (IU/ml)
1	Starch	48.6 ± 0.14	56.6 ± 0.14
2	Maltose	42.3 ± 0.07	51.6 ± 0.14
3	Sucrose	61.3 ± 0.07	70.6 ± 0.56

Values are expressed as Mean ± Standard Deviation.

Table 4: Nitrogen sources of Protease and Lipase

S.No	Nitrogen sources	Protease (IU/ml)
1	Ammonium chloride	70.3±0.07
2	Ammonium phosphate	73.3 ± 0.07
3	Sodium nitrate	60.3±0.07
		Lipase (IU/ml)
1	Urea	57.3 ± 0.07
2	Yeast extract	68.6±0.14
3	Peptone	49.3 ± 0.07

Values are expressed as Mean ± Standard Deviation.

Our study reports similar to Riffel *et al.*, (2002) [15] keratin is a insoluble protein which is found as a major constituent of hair, nail and feathers. Several fungal strains were isolated from natural soil to find more robust and specific keratinolytic enzymes for variety of application The proteolytic and lipolytic potential of this strains were identified as the formation of clear zone on the nutrient agar surface containing casein as a substrate. The zone formation that indicates, the degrading ability of organism due to the presence of lipase and protease enzyme. In chicken feather degradation, the mineral salt liquid medium containing *A.flavus* and *F.solani* separately able to degrade the feathers. Our study reports similar to Abu Sayem *et al.*, (2006) [16] The most significant level growth and production of protease was

supported by pH ranging between 8 and 10. In present study the maximum protease enzyme production was recorded at the pH 11.

CONCLUSION

In the present study fungal colonies were isolated from the feather dumped soil sample. For keratin degradation two different types of fungus *A.flavus* and *F.solani* were selected. Hen feather was used as a substrate for keratin degradation. The production of different enzymes such as protease and lipase from *A.flavus* and *F.solani*. The total amount of protein in enzyme was studied by Lowry's method. Protease enzyme has optimum p^H 7 and temperature 40oC respectively. In the present study, sucrose content (70.6 ± 0.07 IU/ml) present in high amount of protease. Nitrogen source of ammonium phosphate (73.3±0.07) was present in high amount of protease. Protein (2.475) was noted in enzyme. Finally it was concluded that *A.flavus* strain produced high levels of protease, lipase enzymes, compared than *F.solani*. Waste management is the important process to product the environment from the pollution. Hen feather waste materials cause serious environmental problems. So, the waste materials are removed by using the microorganisms. The keratinolytic microorganisms and technologies developed for feather degradation not only remove the waste feathers efficiently from the nature but also make the by-products of the process as a valuable protein supplement. The protein rich, concentrated feather meal can also be used for organic farming as semi-sow release, nitrogen fertilizer.

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