



RESEARCH ARTICLE

Keratinase Activity and Biodegradation Properties of Hyphomycetous Fungi from Fowl Feather

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ABSTRACT

This study was carried out to evaluate the ability of hyphomycetous fungi to degrade fowl feather using its keratinase activity. The soil sample used for the study was collected at random from a poultry farm at Awka. Isolation and identification of the fungi was carried out using baiting technique, serial dilution, colonial morphology and microscopic examination with the aid of a fungi atlas. The keratinase activity of the isolates was carried out in a submerged shaken culture. The protein released in the medium was measured spectrophotometrically. The highest keratinolytic activity was recorded by *Aspergillus niger* (39.70 μ /ml), *Candida* species (29.40 μ /ml), *Aspergillus flavus* (28.10 μ /ml), *Trichophyton megnini* (12.60 μ /ml) and the least being *Aspergillus oryzae* (11.60 μ /ml). The use of feather waste and isolation of the keratinolytic fungi from the soil has been shown to be an ideal and effective method of poultry waste management.

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INTRODUCTION

Feather is one of the epidermal growths that form the distinctive outer covering on the bird. Feather aids in flight, thermal insulation, water proofing and coloration that helps in communication and protection. Poultry feather constitutes the most abundant of keratinous material in nature. The main component of feather is keratin, a mechanically durable and chemically unreactive and insoluble protein, which render it difficult to be digested by most proteolytic enzymes. Due to the insoluble nature of keratin, it is resistant to enzymatic digestion by plant, animals and many know microbial proteases. Therefore, the keratinase producing microorganism has been described having the ability to degrade insoluble keratin in feather (Geethanjali, 2011). Due to the strength and stability of keratin a very few organisms are able to break it down and utilize it. Fungi are an important group of organisms known to produce keratinase when grown on keratin rich substrate (Geethanjali, 2011).

The keratinous waste are largely increasing and accumulating in the environment mainly in the form of feather generated from poultry processing industries. Today it is also becoming a part of solid waste management since it is tough to degrade due to the highly rigid structure rendered by extensive disulphide bond and cross linkage. Keratinase enzymes are widespread in nature and are elaborated by several microorganisms,

most of them isolated from fowl feather. Keratinase in nature have been continuously contributing to valorization of voluminous keratin containing waste in the form of hair, feather, dead birds and animals (Farag and Hasan, 2004). Despite the recalcitrance, keratin waste can be efficiently degraded by a myriad of bacteria, actinomycetes and fungi due to the elaboration of keratinolytic proteases called keratinases (Onifade *et al.*, 1998).

The most keratinolytic group among fungi belongs to fungi imperfecti. Hyphomycetous fungi synthesize variety of hydrolytic enzymes. Since the keratinase of many hyphomycetous fungi have not exploited it is therefore need of the day to exploit them. The machismo of colonization and decomposition of the keratinous substrate have received little attention. The fungi which degrade these substrates completely are termed as keratinolytic. Keratinolytic enzyme are widespread in nature and are elaborated by a numerous microorganisms isolated from different habitats and sources. Worldwide poultry processing plants producing millions of tons of feathers as waste products annually, which consist of approximately 90% of keratin. Feather in spite of being made up of almost pure keratin protein is neither profitable nor environmentally friendly forming a production of high volume with low profit margin. Utilizing poultry feather as a fermentation substrate conjunction with keratin degrading microorganisms or enzymatic biodegradation may be a better alternative to improve nutritional value of poultry feathers and reduce

environmental waste. The accumulation of fowl feathers in natural setting suggests the existence of keratinolytic microorganism. These feather degrading hyphomycetous fungi may contribute to understanding the role of these fungi in the degradation of complex keratinous substrate in nature (Kushwaha and Nigam, 1996).

The objective of this study was to determine the keratinase activity and biodegradation properties of isolated hyphomycetous fungi on fowl feather.

MATERIALS AND METHODS

Collection of sample

Soil sample

Ten Soil samples were collected in a poultry farm at Awka, Anambra State, Nigeria. The samples were collected by removing the surface soil and digging into the ground up to 2 cm. The sample was collected at random using hand gloves and a sterile container.

Feather sample

The feather samples used for this study were collected in a poultry farm at Awka, Anambra State, Nigeria. The sample was collected from 20 fowls using a surgical blade, a hand gloves and a sterile container. Some of the fowl feather collected was grinded into powdery form.

Isolation of fungi

Baiting technique

Baiting technique was used as one of the method for isolation of the fungi. It was done by pouring some quantity of the soil sample into a sterile Petri dish and placing some of the raw fowl feather on the soil sample, and then it was incubated at room temperature for 7 days. Organism yield was culture in Sabourand Dextrose Agar medium containing an antibiotic chloramphenicol to inhibit bacteria growth (Maghraby *et al.*, 1991).

Serial dilution

Using masking tape, 10 sterile test tubes were label as 1 to 10 containing 9ml of distilled water. In a separate test tube the stock was prepared using 1 g of the soil sample in 9ml of distilled water. 1ml of the stock was transferred to the first test tube labeled 1 this represents a 10^{-1} dilution. Using aseptic technique tube 2 was inoculated with 1ml from tube 1, a 10^{-2} dilution and the same follows for 3rd to 10th tube respectively 10^{-5} , 10^{-6} , 10^{-7} was plated in SDA agar for 5 to 7 days (Maghraby *et al.*, 1991).

Identification of fungi

The colony was carefully examined. The rate of the growth, consistency, colour and texture of the surface growth, nature of the reverse side and other peculiar features of the colony were noted and were guide to final identification. Final identification of isolated were based on standard criteria for fungal identification ((Rippon, 1988; Frey *et al.*, 1979; Watanabe, 2002).

Inoculum preparation

The identified fungi subcultured on to sabourand dextrose agar were used to prepare the inocula by suspending the fungi spores in 1 ml of distilled water.

Production of keratinase

This was carried out using a modified method of Burt and Ichida (1999) feather degrading activity by the fungi isolates was done using secondary white feathers of fowl. Fowl feather were thoroughly washed with detergent in warm water followed by washing with acetone over 24 hours. Then the feathers were washed with sterile distilled water and air dried. The keratinolytic properties of the isolated fungi were assessed on a mineral medium containing 1.5 g K_2HPO_4 , 0.025 g $MgSO_4 \cdot 7H_2O$, 0.025 g $CaCl_2$, 0.015 g $FeSO_4 \cdot 7H_2O$, 0.005 g $ZnSO_4 \cdot 7H_2O$ and 1000ml distilled water at PH 6.8-7.0. The media supplemented with 500mg of feather was distributed in 250ml Erlenmeyer flask and autoclaved at $121^{\circ}C$ 15 PSI for 15 minutes. The flasks were inoculated with spore suspension. The spore suspension was prepared from ten day old cultures of the test fungi by adding 5ml of sterile distilled water to heavily sporulating slant. The slant was shaken vigorously and the concentration of the cell was adjusted to 10^5 cell/ ml. The culture suspension of 0.5 ml was inoculated in the flask under aseptic conditions. Three replicate were prepared from the same suspension. The flasks were incubated at $27^{\circ}C$ with manual shaking daily for 15 days.

The feather was considered to be degraded when pieces 0.5mm-2mm or smaller remained. The culture filtrate was collected, centrifuged at $2000 \times g$ for 30minutes to remove all spore. The supernatant was filtered through what man No 1 filter paper. The preparation was used as crude enzyme to study the changes that associated with biodegradation such as pH, keratinase activity and protein released during keratin substrate in order to monitor the degradation rates.

Determination of pH

The pH of the sample was determined using pH meter after standardization with a phosphate buffer at 7.0 for every five days interval.

Determination of protein in the culture medium due to keratin substrate degradation

The concentration of soluble protein was determined using the modified method of Kumar *et al.* (2012). 0.9 ml Biuret reagent was mixed with 0.3 ml of the test sample. The mixture was allowed to stay for 5minutes. The absorbance was read at 540 nm using Bovine serum albumin (BSA) as standard.

Keratinase activity assay

The assay of keratinase activity was based on the method of Kumar *et al.* (2012) with little modification. Twomilliliters of 0.05 mol/l Tris/HCL buffer (pH= 7.5) and 1.0 ml of the enzyme solution was incubated with 10 mg feather powder for 1 hour at $37^{\circ}C$, with constant agitation in a water bath. The enzyme reaction was stopped by the addition of 2.0 ml 10% trichloroacetic acid (TCA) and the sample was centrifuged for $1450 \times g$ for 30 minutes, the absorbance of the supernatant was determined at 280nm against a control. The control was prepared by incubating the enzyme (keratinase) solution with 2.0 ml TCA with the addition of 10 mg feather powder.

Table 1: Colonial and microscopic identification of hyphomycetous fungi

Colonial morphology	Microscopy	Isolate
Rapid growth, powdery, white, turning yellow, becoming dark to deep brown	Septate hyphae presence of free spore on a spore stalk or conidiophores	<i>Aspergillus niger</i>
Moderate growth, pale pink to violet, suede-like, reverse is red	Presence of pyriform, microconidia	<i>Trichophyton megninii</i>
Rapid growth, pale greenish, yellow or dull brownish shade	Long conidiophores, radiating or loosely columnar conidial head. subspherical vesicle.	<i>Aspergillus oryzae</i>
Rapid growth, downy to Powdery yellow green walled	Radiating conidial head. Rough walled conidiophores.	<i>Aspergillus flavus</i>
Grows immediately, Creamy white	Rough Conidia in chain. Uniseriate and Biseriate phialide Forms pseudohyphae with enlarged colored blast spore	<i>Candida</i> species

RESULTS

Five hyphomycetous fungi were recovered from the soil samples. These include: *Aspergillus niger*, *Trichophyton megninii*, *Aspergillus oryzae*, *Aspergillus flavus* and *Candida* species (Table 1).

The five hyphomycetous fungi identified were able to degrade and utilize the fowl feather as substrate thus secreting the enzyme keratinase after 15 days of incubation.

The five hyphomycetous fungi isolated yielded variable amount of protein at different pH levels. The pH values and protein content of the medium were as follows *Aspergillus flavus* 9.6 and 35.45 mg/ml, *Candida* species 9.7 and 37.04 mg/ml, *Aspergillus oryzae* 8.6 and 26.96 mg/ml, *Trichophyton megninii* 8.9 and 27.63 mg/ml, *Aspergillus niger* 9.8 and 41.91 mg/ml respectively as shown in Table 2.

The five different fungi identified demonstrated keratinolytic activities as follows:

Aspergillus flavus 28.10 μ /ml, *Candida* species 29.40 μ /ml, *Aspergillus oryzae* 11.60 μ /ml, *Trichophyton megninii* 12.60 μ /ml, *Aspergillus niger* 39.70 μ /ml. One unit (μ /ml) of keratinolytic activity was defined as an increase of corrected absorbance of 280 nm (A_{280}) with the control for 0.01 per minute under the conditions described above.

Table 2: Physicochemical parameter of the keratinized fungi during biodegradation activity

Organism	pH	Protein (mg/ml)
<i>Aspergillus flavus</i>	9.6	35.45
<i>Candida species</i>	9.7	37.04
<i>Aspergillus oryzae</i>	8.6	26.96
<i>Trichophyton megninii</i>	8.9	27.63
<i>Aspergillus niger</i>	9.8	41.91
Control A	7.7	–
Control B	7.8	–

Key: A = Medium + Organism; B = Medium + keratin

Table 3: Keratinolytic activity of fungal organism

Organism	Activity (μ /ml)
<i>Aspergillus flavus</i>	28.10
<i>Candida species</i>	29.40
<i>Aspergillus oryzae</i>	11.60
<i>Trichophyton megninii</i>	12.60
<i>Aspergillus niger</i>	39.70
Control A	–
Control B	–

DISCUSSION

Keratinophilic fungi have been frequently isolated from soil, where they colonize various keratinous

substrates, degrade them and add the mineral content to the soil. The keratinophilic hyphomycetous fungi use the proteins as a sole source of carbon and nitrogen. Keratinophilic hyphomycetous fungal forms remove excess nitrogen through intensive deamination and ammonia production which results from alkaline conditions, which are prevalent during keratinolysis. Keratinolytic fungi metabolise sulphur since keratin is sulphur-rich substrate. The fungi would release the enzyme keratinase that degrade β -keratin disrupting the disulphide bonds. In the present study, five different species of keratinolytic fungi were isolated from the soil and feather samples. These included: *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton megninii* and *Candida* species. This is in accordance with previous findings of Kim (2003); Farag and Hassan, (2004); Thanna *et al.*, (2011) and Ingle *et al.*, (2012).

All the five fungi were very effective in synthesizing the keratinolytic enzyme as well as in feather degradation. The effect of pH on the degradation of feather by these fungi was tested. The maximum degradation was within the pH range of 9.8 to 9.7. The pH increases with increase in the amount of protein released this agrees with (Ingle *et al.*, 2012 and Mukesh *et al.*, 2010). The keratinolytic nature of the examined fungi toward keratin may be related to their ability to degrade the keratin thus liberating protein as reported in other studies (Kunert and Krajei, 1981; Mukesh *et al.*, 2010).

The hyphomycetous fungi have emphasized their specific properties of fungal enzyme able to digest keratin, a substrate extremely resistant to the action of physical and chemical agent. The ability of these isolated hyphomycetous fungi to produce keratinase which is able to degrade the feather shows that these organisms are potentially pathogenic to human skin causing superficial mycoses because it contains keratin which is the same component as the feather (Mukesh *et al.*, 2012).

This present study has shown that *Aspergillus niger* is the most active keratinolytic hyphomycetous fungi among the five hyphomycetous fungi isolated. This species released the highest protein from the fowl feather followed by *Candida* species, *Aspergillus flavus*, *Trichophyton megninii* and *Aspergillus oryzae*. This shows their level of pathogenicity respectively. Thus, these fungi and their keratinase offer an effective solution for biotechnological potential applications in feather waste management.

Conclusion

Some feather degrading hyphomycetous fungi were isolated from poultry soil. These organisms were able to

grow in basal medium with feather meal as their primary source of carbon, nitrogen, sulphur and energy thus producing keratinase in order to utilise this substrate (fowl feather). These keratinase activity and biodegradation properties discovered from these fungi enables them to become natural degrading machine which serves as a method of environmental waste management.

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