IFERP

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 4, Issue 3, March 2019

Isolation of Keratinolytic Microorganisms And Estimation of Keratinolytic Activity

[1] R.V.Madhumitha Indu, [2] Dr.P.Vanitha Pappa
[1][2] Research scholar, [2] Assistant professor
[1][2] Department of Zoology, Rani Anna Government College for women, affiliated under Manonmaniam Sundaranar University, Abisekapatti, Tirunelveli, Tamil Nadu, India.
[1] madhumithaindu@gmail.com, [2] biovanitha@rediffmail.com

Abstract: **Chicken feathers are the most abundant keratinous biomass in the world. Only 60-70% of the poultry slaughterhouse products are edible. The slaughterhouse by-products contain keratin which forms more than million tons of waste worldwide annually. Disposal of the huge and increasing volume of waste feathers, presents as a major concern for poultry industry. On the other hand, energy and material recovery of this valuable protein source is an important issue for organic solid waste treatment and bioenergy generation. The high protein content of poultry feather makes it an excellent raw material for various utilizations. Dried feather contains 91% proteins and with 0.2 methane potential (m 3 kg-1 VS added) and 0.05 methane potential. The keratin-content of the feather has the difficulty in pre-treatment before utilisation. Fungal isolates: Aspergillus flavus, Aspergillus niger and Fusarium species are found to produce keratinase enzyme, which can be beneficially used for pre-treatment of feather. Different types of fungal isolates which degrade keratinous substrate are screened using feather bait technique. The isolates were then cultured in feather meal medium to induce keratinase enzyme production. The activities of enzyme produced by different isolates were estimated using spectroscopic technique. The potential feather degrading enzyme is then analysed. The treated keratinous waste can be used economically in bio gas production, as fortified food for animals and also as bio fertilizer for plants. Recycling of the slaughterhouse feather and by-products can solve three main problems: disposal of harmful materials, producing of renewable energy and soil nutrient. Feather waste-this difficultly disintegrating material produced in large amount-finds an environmentally friendly way of utilization.**

Keywords: - **Feather bait technique, Keratinolytic fungi, estimation of keratinase activity, potential keratinolytic fungi_Aspergillus flavus**

I. INTRODUCTION

The Poultry Business in India is one of the important contributors to the economy of rural and semi-urban India. India is the fifth largest producer of eggs and ninth largest producer of poultry meat amongst all the countries. The common method of waste disposal from such industries is open dumping. Huge numbers of municipal solid waste (MSW) are generated through various food industries among which meat industry generate a waste such as feather, horns, hoof, hair etc. Feathers are generated each year by poultry processing unit, creating a serious solid waste management problem.

Feather is composed of keratin, a protein [1] that makes feather recalcitrant to common proteases making its degradation difficult [2].The mechanical stability and high resistanace to proteolytic degradation of keratin is due to the presence of disulphide bond and cross linkages [3]. Keratinase producing micro organism has the ability to degrade these keratinous substance[2]. Keratinolytic enzyme (Keratinase) is produced by diverse microorganisms belonging to the eucarya, bacteria and archea domains in the presence of keratin-containing substrate. Many micro organism especially that belongs to fungi have high keratinolytic activity including the following genera: Chrysosporium, Aspergillus, Alternaria, Trichurus, Curvularia, Cladosporium, Fusarium, Geomyces, Gleomastis, Monodictys, Myrothecium Paecilomyces, Stachybotrys, Penicillium and Doratomyces. However, the isolation of filamentous fungi that efficiently degrades keratinous wastes is very interesting.

The present day study aims in production of potential Keratinase enzyme by using biological techniques for faster degradation of keratin containing substrate in eco friendly manner and development of effective processes for production of energy saving materials from poultry waste. The high protein content of feather makes it an excellent raw material for biogas production and supplement as animal feed. The high nitrogen content makes it a source of bio fertilizer.([3],[4]).

The utilization of keratinous wastes as a fermentation substrate (carbon and nitrogen sources) by keratin-degrading fungi offers a feasible microbial technology for obtaining keratinolytic enzymes. These enzymes, besides waste

All Rights Reserved © 2019 IJSEM

feather elimination, could find their application in the food industry, manufacturing of textiles, biodegradable films, glues and foils, cosmetics, leather industry and nitrogenous fertilizer for plants

The enzymatic biodegradation may be a better alternative to improve their nutritional value and offers cheap and mild reaction conditions for the production of valuable products: amino acids, such as methionine, lysine and tryptophane[4]. Hence, feather waste is also utilized as a source of nitrogen for plants or as a dietary protein supplement for animal feedstuffs ([5],[6])

This study is a report on isolation, screening of keratinolytic fungi for biodegradation of keratinous wastes using native isolates and estimation of potential keratinase.

II. MATERIALS AND METHODS

1. Isolation of keratinolytic fungi

1a.Feather bait method:

\Keratinophilic fungi were isolated by hair baiting technique of Vanbreuseghem. Sterile Petri dishes half filled with the soil samples and moistened with sterile distilled water and were baited by burying sterile keratinous bait in the soil. These dishes were incubated at room temperature and examined regular period

1b.Isolation of pure cultures by Hyphal Tipping:

In order to achieve pure fungal culture, a technique called hyphal tipping was performed. Using the dissecting microscope the species are viewed at high magnification. An individual strand of fungal hypae is located. After flaming the scalpel, the ends of the hyphal strand were cut (about 1 mm from end) and are transferred to Potato Dextrose Agar plate. The agar plates are incubated for 48 hours

2. Sporological identification:

Fungal isolates were stained with Lacto Phenol Cotton Blue and viewed in binocular research microscope and micrographed. Structure of spores, Conidia, the topology of attachment of spores with conidia and hypae were studied.

3. Determination of Keratinolytic Activity

3.1 Preparation of Chicken Feather Powder

Poultry feather was cut into small fragments washed extensively with water and dried in a hot air oven at 40°C for 72 hours. To prepare feather powder, the feathers were pulverized and passed through a small mesh grid to remove coarse particles.

3.2 Inoculum Preparation

Spore suspension of the fungal isolates was prepared by adding 10 mL of sterilized water to 7 days old fungal isolates growing on plates of potato dextrose agar. The final concentration of the spore suspension was adjusted to about 2x106 mL-1

3.3 Feather meal preparation:

Feather meal medium contain (g L^{-1}); $MgSO_4.H_2O$; 0.5, KH_2PO_4; 0.1, $FeSO_4.7H_2O$; 0.01 and $ZnSO_4.7H_2O$; 0.005, the pH was adjusted to 7.5. The medium was supplemented with chicken feather powder as a sole source of carbon and nitrogen [7].

3.4 Degradation of feather by Isolated Fungi:

In 100 mL conical flasks, 50 mL of the feather meal medium along with 1.5 g of poultry feather powder was added separately in each flask then autoclaved. After cooling, the flasks were inoculated by 5 mL of spore suspension and incubated under shaking (120 rpm) at 28°C for 7 days. At periodic intervals, final culture pH was determined and the cultures containing the hydrolysates were centrifuged (8000 rpm) and filtered through muslin cloth. The filtrate was recovered to determine the keratinase activity

3.3 Keratinolytic Activity:

Keratinase activity was assayed by the modified Yu et al. (1968). 20 mg of chicken feathers powder were suspended in 3.8 mL of 100 mM Tris-HCl buffer (pH 7.8) to which 300 μL of the culture filtrate (enzyme source) was added. The reaction mixture was incubated at 37°C for 1 h. After incubation, the assay mixture was dipped into ice-cold water for 10 min and the remaining feathers were filtered out. Then the absorbance of the clear mixture was measured at 280 nm -400nm using UV-spectrophotomete.

4. Rate of Degradation of feather

The potential isolate is checked for increase in rate of the feather solubility. The rate of the feather solubility was determined using the formula [(dry weight of the residual feather/ initial dry weight of the feather)*100]

III. RESULT

1. Isolation of keratinolytic fungi

1a.Feather bait method:

Colonies of fungi was seen around the feather. Three different colony morphologies are found as shown in Figure1.1

All Rights Reserved © 2019 IJSEM

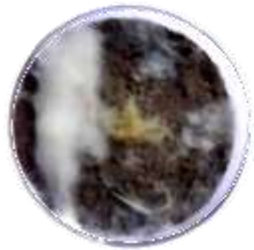

Figure 1.1 Culturing Fungi using Feather bait method

1b.Isolation of pure cultures by Hyphal Tipping:

QThree different morphological colonies of fungi are formed on the PDA plates. First isolate showed granular, velvety, and green colour colonies. Second isolate showed colonies which were white to pale yellow; the reverse was buff or yellow-gray. Third isolates were cottony, white becoming dark-grey and colonies. Each colony were designated as S1, S2, S3 respectively, as shown in figure 1.2.

Figure 1.2A (S1)   Figure 1.2B (S2)   Figure 1.2C (S3)
Figure 1.2 Plates showing pure culture of fungal Isolates

2. Sporological identification

Figure 2 Micrograph of Three different fungal isolates.S1 beared conidiophores with heavy walled, hyaline, roughened, and just below the apex. It has series of conidia-bearing cells(supporting cells).Conidia were dome-shaped. S2 beared Conidiophores that were smooth, hyaline. Apices are spherical. Conidia are spherical. S3 beared abundant microconidia. They were hyaline, one-celled, oval, and slightly flattened at each end. Macroconidia are formed infrequently. They were hyaline, delicate with thin walls, curved to almost straight, and had a foot-shaped basal cell.

These characters are compared with "A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain" by S S Navi et al.and identified the species S1, S2, S3 is identified as Aspergillus flavus, Aspergillus niger, Fusarium species respectively.

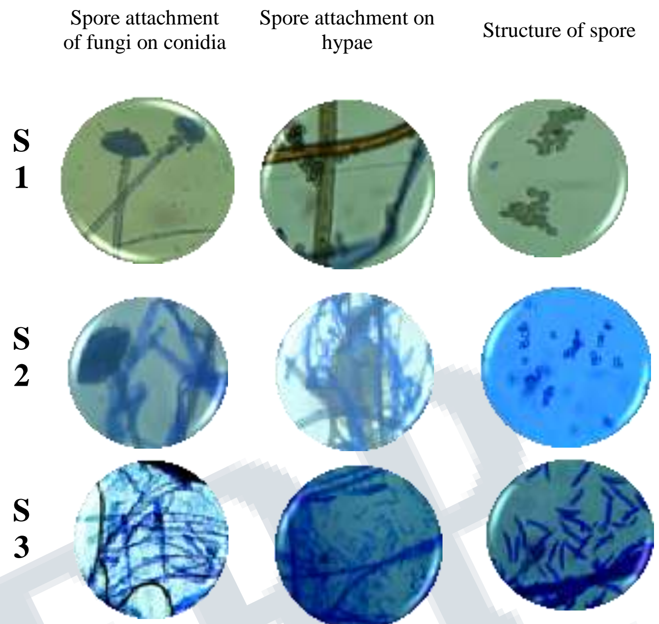

Figure 2 Micrograph of Three different fungal isolates

3. Degradation of Keratinous substrate by the Isolated Fungi

Table 1 shows the Absorbance of different isolates estimated. By analysing the present day study the enzyme produced by fungal isolate Aspergillus flavus is found to hold the maximum keratinolytic activity. Minimum activity is seen in Fusarium species

Figure 3.1 Graphical representation keratinolytic activity of *Aspergillus flavus*	**Figure 3.2 Graphical representation keratinolytic activity of *Aspergillus niger***	**Figure 3.3 Graphical representation keratinolytic activity of *Fusarium species***

Figure 3: The graphical representation of absorbance observed in fungal isolates

All Rights Reserved © 2019 IJSEM

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 4, Issue 3, March 2019

4. Rate of Degradation of feather

Solubility rate of feather determines the deradation rate of feather. The initial rate of feather is found to be 1.0 gram And after 50 days of incubation it is found to be 0.28 grams. Hence by applying the formula the rate of feather solubility is 28% more than in normal condition.Figure 3 shows the plate containing feather before and after incubation with Aspergillus flavus for 50 days

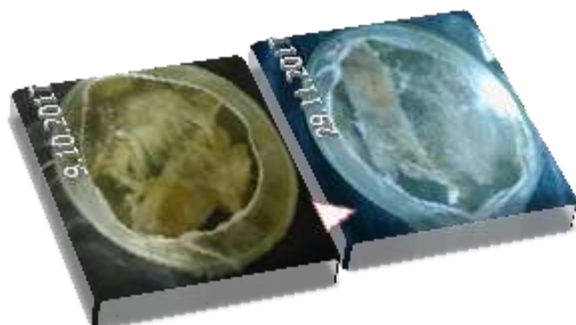

Figure 3 The plate containing feather before and after incubation with Aspergillus flavus for 50 days

IV. DISCUSSION

Keratinophilic fungi are important ecologically and recently have attracted the attention throughout the world. My present study shows the role of keratinophilic fungi play a significant role in the natural degradation of keratinized residues. It was revealed that the keratinolytic fungi Aspergillus flavus, Aspergillus niger, and Fusariumspecies are commonly found in poultry waste dumping area in Tirunelveli. Keratinophilic fungi are important ecologically as they play a significant role natural degradation of keratinized residues[8].

Hedayati et.al., 2005 [9] observed two major techniques that have been used for the qualitative and quantitative isolation of these fungi from soil: Surface soil dilution technique(SSDP) and Feather baiting technique (FBT). FBT which is most common and reliable method used in the present investigation and yielded two groups of keratinophilic fungi (Aspergillus species, and Fusarium species).

The different organisms showed different optimum value of microbial growth in the given feather meal medium which makes the fungus to assimilate the keratin as a sole carbon source which increases the percentage of solubilization, as the keratinolytic organisms.

The Keratinase activity was expressed as one unit of the enzyme corresponding to an increase in the absorbance value 0.01 h-1.The enzymatic activity of isolated cultures showed different values. In spectroscopic analysis at (280nm), it is found that the enzyme secreated by Aspergillus flavus has the absorbance value of 3.5, the enzyme secreated by Aspergillus niger has the absorbance value of 3.2 and the enzyme secreated by Fusarium species has the absorbance value of 2.8 units. The results of the work done by Gampa Ramakrishanaiah et.al., in 2013 [10] coincides my results. Table 1 shows the Absorbance of UV light at 280 nm wavelength by keratinase enzyme produced by three different fungal isolates. Figure 4 Graphical representation of shows the Absorbance of UV light at 280 nm wavelength by keratinase enzyme produced by three different fungal isolates.

Table 1 The Absorbance (at 280 nm) by keratinase enzyme keratinase enzyme produced by three different fungal isolates

Name of the isolate	Aborbance (at 280 nm)
Aspergillus flavus	3.5
Aspergillus niger	3.2
Fusarium species	2.8

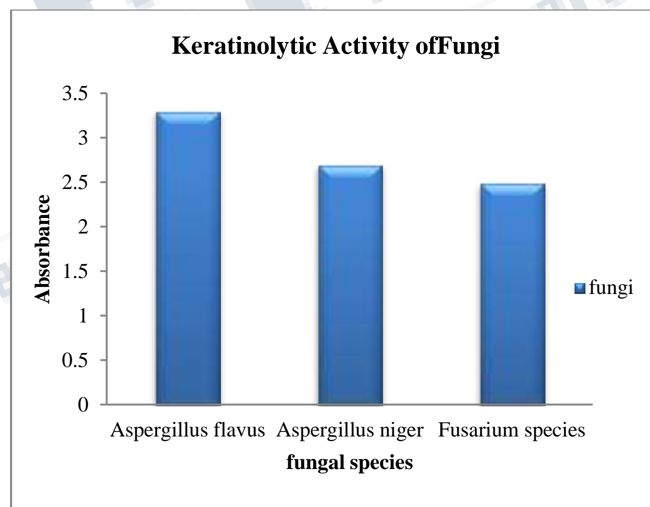

Figure 4 Graphical representation of Absorbance (at 280 nm) by keratinase enzyme produced by three different fungal isolates

Among three fungal isolate, the highest enzyme activity is seen in Aspergillus flavus, lowest enzyme activity is seen in Fusarium species. The work done by Helena et.al., 2005 [11] supports my results. In Helena et.al., work the outstanding enzyme activity was found in Keratinases produced by Aspergillus flavus strains.

All Rights Reserved © 2019 IJSEM

IFERP
ISSN (Online) 2456 -1304
International Journal of Science, Engineering and Management (IJSEM)
Vol 4, Issue 3, March 2019

V. CONCLUSION

Chicken feathers are available in large quantities around the world causing environmental challenges. Bioremediation is the permanent solution for managing the feather waste. This study will designate that feather is economical, readily available and environmental friendly natural resource that offers a promising prospect in agriculture as an organic fertilizer, animal feed or feed supplement in animal husbandry and an effective raw material for biogas production, if applied at recommended rates and time

REFERENCES

1. Acda.M.N,2010; "waste chicken feather as reinforcement in cement bonded composites," Philippine journal of science , vol.139,no.2, pp.161-166.
2. Cai C.G, Lou B.G and Zheng X.D,2008; " Keratinase production and keratin degradation by a mutant strain of Bacillus subtilis Journal of Zhejiang University Science.B, Vol.9 no.1, pp 60-67.
3. Wawrzkiewicz, K., Wolski, T. and Lobarzewski, J. (1991) Screening the keratinolytic activity of dermatophytes in vitro. Mycopathologia 114, 1–8.
4. Kanchana.R, 2012; "Farm waste recycling through microbial keratinases," Journal of Applied Sciences in Environmental sanitation, vol.7, no.2,pp.103-108.
5. Ramnani.R, 2006; "Microbial keratinases and their prospective applications" an overview.Appl Microbial Biotechnol vol.70:21-33.
6. Williams C, Grimes J, Mikkelsen R,1999; " The use of poultry litter as co-substrate and source of inorganic nutrients and microorganisms for the ex-situ biodegradation of petroleum compounds". Poultry science1999. Vol.78(7):956-64.
7. Elmayergi HH, Smith RE1,971.; "Influence of growth of streptomyces fradiae on pepsin –HCL digestibility and methionine content of feather meal". Can J Microbial;vol.17(8): 1067-72.
8. Sharma R, Rajak RC,(2003); Keratinophilic fungi: Nature's keratin degrading machines! Their isolation identification, and ecological role. Vol:35 No.4, pp.28-40
9. Hedayti MH, Mohseni-Bandpi A, Moradi S, 2005; A survey on the pathogenic fungi in soil samples of plotted plants from sari hospitals. Journal of Hospital Infection. Vol:58 pp.59-62
10. Gampa Ramakrishanaiah, Mustafa S.M, Srihari.G,2013;"Studies on keratinase producing fungi isolated from poultry waste and their enzymatic activity" Journal of Microbiology research vol.3(4):148-151.
11. Helena Gradisar Jozica Friedrich, Igor krizaj, Roman Jerala, 2005; "Similarities and specificities of fungal keratinolytic proteases: Comparison of keratinases of Paecilomyces marquandii and Doratomyces microspores to some known proteases". Applied and environmental microbiology vol.71 no.3420-3426

All Rights Reserved © 2019 IJSEM