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Management of Poultry feather waste using keratinolytic Fungi

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Abstract

The poultry industry in Maharashtra State of India has developed in leaps and bounds in last few decades. This had lead to increased problem of feature disposal. Generally the feathers take months together for natural degradation.

A systematic study for the degradation and management of these poultry waste was undertaken. The soil samples from poultry farms were subjected to the microbiological analysis for isolation of feather degrading microbes. The screening of the fungi isolated from the soil of poultry farms for synthesis of keratinolytic enzymes on feather based medium was done. Of the 12 fungi isolated Chrysosporium tropicum, C. keratinophilum, Microsporium cannis, Trichophyton verrucosum, T. equinum and some non sporulating forms were found to be most dominant. These isolated fungi showed appreciable keratinolytic activity. All the strains exhibited keratinolytic enzyme activity.

These strains were further used for degradation of feathers. They were used in mixed culture. After 40 days of incubation of the feathers, this mixed culture initially degraded the feathers very fast which gradually decreased with increase in incubation period. The degradation was optimum at 35 °C within the pH range of 6.0 to 7.0.

Utilizing the feather waste available in the region and isolating the keratinolytic fungi from the indigenous soil proved to be an ideal system in terms of biodegradation and poultry feather waste management as well.

Keywords: *poultry feather waste, keratinolytic fungi, degradation*

Introduction

Concerns over environmental pollution and degradation of ecosystem have assumed prime significance owing to increase in the accumulation of wastes from industries; agriculture and poultry. Solid wastes and chemicals have mainly caused the land pollution. One of the major pollution problems has been the disposal of solid waste materials from poultry farm and animal manure, agricultural wastes and industrial wastes.

Annually more than 20,000 tons of feathers are produced as waste by poultry farming and discharged into the surrounding environment. Huge quantities of feathers bring about serious disposal problems. The feathers discharged into the environment are not decomposed into the soil because of the presence of an insoluble fibrous protein known as keratin. Microorganisms could minimize regulatory problems of uncontrolled accumulation of waste feathers by degrading the keratin present in feathers.

Feathers contain β -pleated sheets of keratin twisted into microfibrils (PAULING AND COREY 1951A, B; BRUSH 1978) and are unusually resistant to biological degradation (GODDARD AND MICHAELIS, 1934, PARRY et al. 1977, LIN et al. 1992). A few species of fungi (PUGH 1964, 1965; HUBALEK 1976, 1978) were known to degrade feathers.

Keratinolytic activity has been reported in many microorganisms like actinomycetes, bacteria and fungi (YA PENG 2007, LIN et al. 1992). Keratinases from fungi have long been well known (AWASTHI 2011). Keratinophilic fungi are a unique group, these fungi are generally imperfect fungi and members of ascomycetes. Some of the fungi also occur in the plumage of a few species of birds, whereas others occur on the bill, in the throat, or in old nests (PUGH 1964, 1965; HUBALEK 1976, 1978). The most appropriate site for recovery is poultry farm soil where feathers of birds are added continuously to the soil, which leads to enrichment of such slow growing fungal forms. Keratinolytic

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enzymes possess major applications in environmental biotechnology and pharmaceutical industries (ANITHA 2013)

Material and Methods

Isolation of keratolytic fungi

The keratinolytic nature of these fungi makes it possible to isolate them from soil by implanting hair, the 'hair baiting' technique initially developed by VANBREUSEGHEM (1949) was used for isolation of these fungi. The organisms used in the study were also isolated from soil collected from yards of poultry farms at Nanded, Maharashtra. Fungi were isolated and enumerated by serial dilution method after growth on the SDA medium (Sabouraud Dextrose Agar). The fungal isolates were identified comparing the characteristic with already described ones (CURRAH, 1985, 1988).

Substrate for enzymatic studies

Feather waste based medium was used for isolation of Fungi and production of keratolytic enzymes. Defatted dried feather pieces were supplemented in SDA medium.

Enzyme activity and protein determination

The keratolytic activity was monitored as describe previously (SANGALI AND BRANDELLI, 2000). 100 µl of enzyme extract was added to 400 µl of 10 mg ml-1 azokeratin in 0.1 M phosphate pH 8.0. The reaction mixture was incubated for 15 min at 50ºC and the reaction was stopped by addition of trichloroacetic acid making a final concentration of 10% (w/v). Absorbance was recorded at 440 nm following centrifugation at 10,000 g for 5 min. One unit of activity was the amount of enzyme that was required for change of absorbance of 0.01 at 440 nm in 15 min at 50ºC. The protein concentration was measured by the Folin phenol reagent method (LOWRY et al. 1951).

Degradation of feathers

Feather degrading activity by the fungal isolates was done using secondary feathers of chickens. The feathers were placed in different test tubes and 10 mL medium was added to each tube. All tubes were sterilized at 15 lbs pressure for 20 min. a loopful of fungal growth was suspended in sterile saline. The turbidity of the saline suspension was adjusted to 0.5 MacFarland standard, which corresponds to about1 50,000 cells/mL. Two drops of this suspension (ca. 0.1 mL) were placed in a test tube of feather media containing the feather. Similarly flask experiments were also carried out. 50ml of Mineral medium was poured in 250ml of conical flasks and sterilized at 15lbs for 20 minutes. The culture suspension of 5ml was inoculated in the conical flask under aseptic conditions. Later the 1000 mg feather pieces of fowl and Broiler were added aseptically in the flasks.

Three replicate were prepared from the same suspension Tubes were placed in a rack on a shaker that rotated at 175 rpm and incubated at 50 C. All tubes were checked daily for 40 days. The feather was considered to be degraded when pieces 0.5 mm 2 or smaller remained. The biochemical changes associated with biodegradation was evaluated by analyzing culture filtrate regularly during complete degradation of feathers. (EDWARD AND ICHIDA, 1999)

Growth conditions

The inoculum was prepared by streaking a SDA supplemented with feather plate with cells from a stock culture of isolated strain. A single colony was transferred to 10 ml of feather meal broth incubated at 30ºC until the cell density reached about 108 cells / ml. Then 1ml was transferred to 500 ml Erlenmeyer flasks containing 100 ml of feather keratin broth (washed, whole feathers are used instead of feather meal) and cultivated in a shaker at 180 cycles/ min for desired times. The culture supernatants were used for assays of keratolytic activity.

The influence of temperature on growth and production of protease and subsequently on the degradation of feathers was studied at 25ºC, 30ºC, 37ºC and 42ºC. Ketatinase production was also investigated in feather keratin broth with initial pH adjusted within 3.0 to 8.0. The cultivation was carried out at 30ºC. When different protein sources were used as substrates, a concentration of 10 g l-1 was used, replacing the raw feathers. All experiments were done in triplicate.

Results and Discussion

Degradation of ecosystem and environment pollution has assumed significance owing to increase in the accumulation of wastes from industries, agriculture and poultry. Every year several tons of feathers are produced as waste by poultry farming. Biodegradation could minimize regularly problems of uncontrolled accumulation of waste feathers. The major component of feather is keratin

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which is an insoluble structural protein. Keratin is highly resistant to hydrolysis by weak acids, alkalis, ethanol or salt solution. The durability of keratin is due to cross-binding of closely packed polypeptide chains in which cystine molecules are held together by disulphide bonds.

However, the kerationophilic 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 fungi use the proteins as a sole source of carbon and nitrogen. Keratinophilic fungal forms remove excess nitrogen through intensive deamination and ammonia production resulting in alkaline conditions, which are prevalent during keratinolysis. Metabolism of sulphur is another of key properties, since keratin is sulphur – rich substrate. The fungi would release the enzymes Keratinases, which would degrade the keratin β disrupting the disulphide bonds.

The ecological relationships between birds and feather-degrading fungi are poorly studied. Among 470 European birds representing 41 species (PUGH 1965), ground-foraging species had a much higher incidence of keratinolytic fungi (*Arthroderma curreyi, A. quadrifidum, Chrysosporium spp.* and *Ctenomyces serratus*) in their plumage than did foliage-gleaning insectivores HUBALEK (1976) examined 502 birds and 367 nests of 90 European species and found that *Arthroderma curreyi, A. quadrifidum* and *Ctenomyces serratus* were most frequent on the plumage of polyphagous, ground-foraging birds, whereas *Chrysosporium tropicum* was most frequent on the plumage and in the nests of birds that live in aquatic or forest habitats. Keratinolytic microorganisms occur most frequently in the plumage of ground-foraging birds and less frequently on species that forage above the ground. The latter species pick up the microorganisms either through their infrequent contact with the ground (e.g.when gathering nest material or dust bathing) or through contact with the aerial spores of bacilli and fungi.

All the dominant strains exhibited strong keratolytic activity. The selected strains showed secretion of extracellular keratinase (Figure 1). However, there was variation among the quantity of keratinases synthesized by these selected strains. The maximum enzyme activity was shown by *Trichophyton. equinum* (75 U/ml), *Chrysosporium tropicum* (74 U/ml), followed by *Trichophyton verrucosum* (68 U/ml), *Chrysosporium keratinophilum* (65 U/ml) and *Microsporium cannis,* (64 U/ml).

Most strains tested for degradation of feathers shows degradation of keratin. A rapid response was exhibited by the dominant strains, however, isolates of keratinolytic species showed little variation in degradation of the feathers. keratin degradation was recorded in 42 or 44 days by most of these species tested but when a combination all the five strains was tested the degradation of feather was in 40 days only. Density of growth did not appear to be well correlated with the degree of degradation; most of the strains tested showed visible growth. But, the visual assessment of mycelial production was not considered to be informative for the scoring of degradation of feathers (Figure 2).

The effect of pH on degradation of the feather by selected strains was tested. The maximum degradation was within the pH range of 6 to 7 where as the higher and lower pH altered the time of degradation. With in the range 6 - 7 pH the complete degradation of the feather took place within 44 days by the individual strains. However the combination all the five strains showed the degradation within 40 days (Figure 3).

Similarly, the effect of temperature on degradation of the feather by selected strains was also studied. Maximum degradation was recorded at 35 °C where as the higher and lower temperature altered the time of degradation. At 35 °C the complete degradation of the feather was within 42 days by the individual strains but the combination all the five strains showed the degradation within 40 days (Figure 4).

Thus the feather waste was degraded with efficiently using selected keratolytic fungi alone or in combination. It can be concluded that the culture of keratolytic fungi due to synergistic effects accelerated the biodegradation of the feather waste reducing the pollution problem associated with its disposal.

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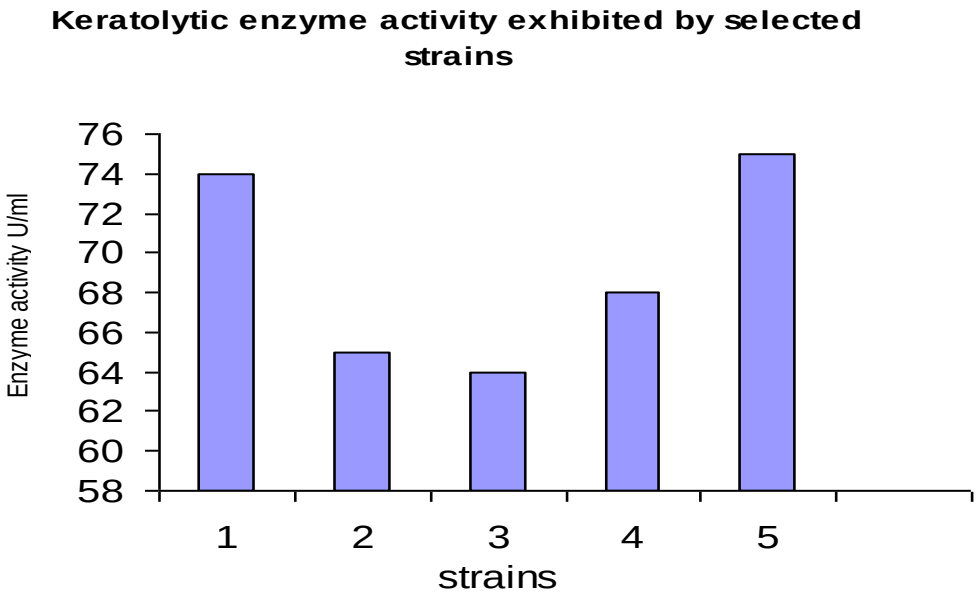

Figure 1 Keratolytic enzyme activity exhibited by selected strains
(1 Chrysosporium tropicum, 2 C. keratinophilum, 3 Microsporium cannis,
4 Trichophyton verrucosum 5 T. equinum)

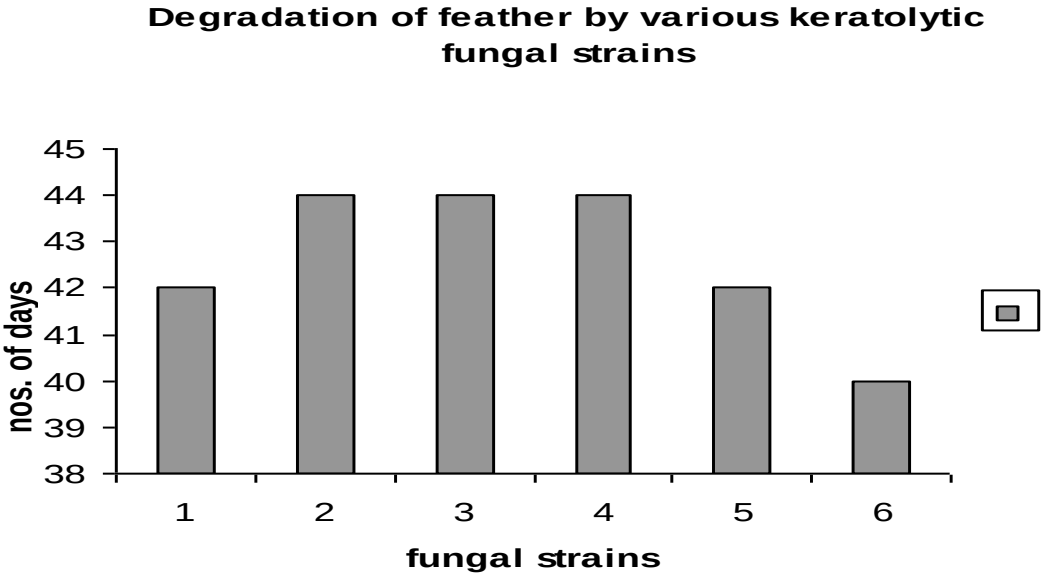

Figure 2 Degradation of feather by various keratolytic fungal strains
(1 Chrysosporium tropicum, 2 C. keratinophilum, 3 Microsporium cannis,
4 Trichophyton verrucosum 5 T. equinum)

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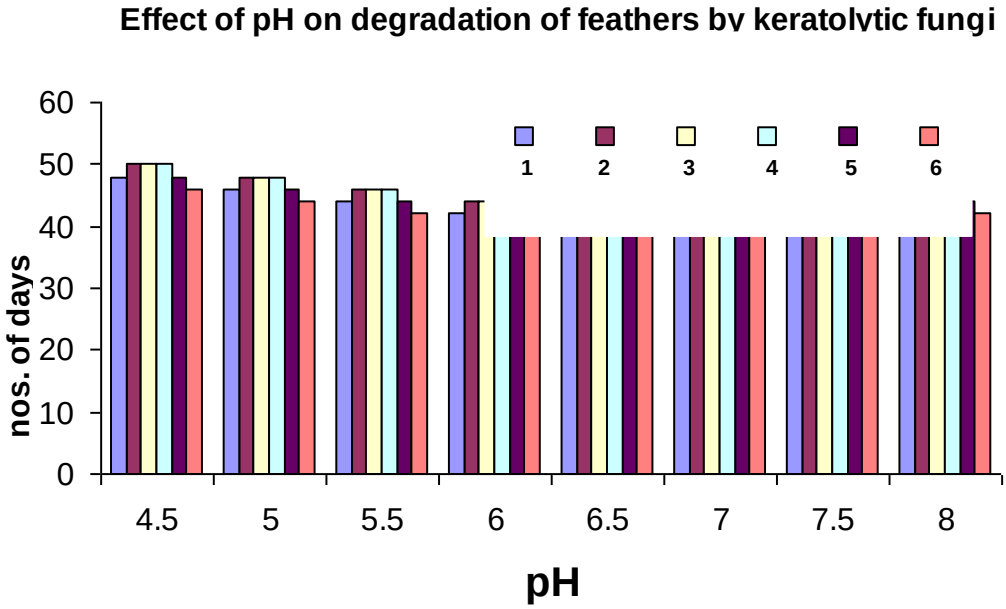

Figure 3 Effect of pH on degradation of feathers by keratolytic fungi
(1 Chrysosporium tropicum, 2 C. keratinophilum, 3 Microsporium cannis,
4 Trichophyton verrucosum 5 T. equinum)

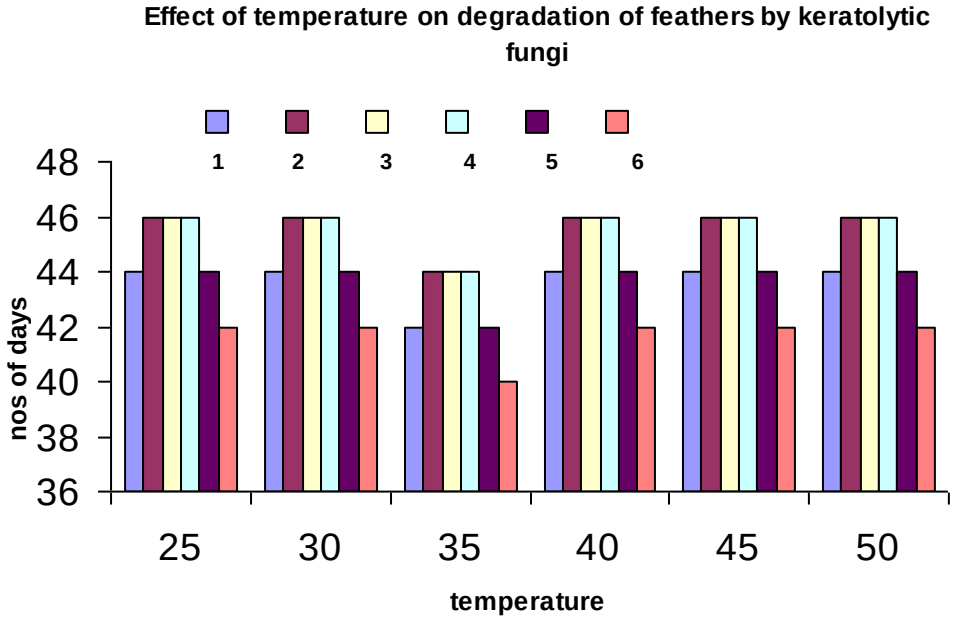

Figure 3 Effect of temerature on degradation of feathers by keratolytic fungi
(1 Chrysosporium tropicum, 2 C. keratinophilum, 3 Microsporium cannis,
4 Trichophyton verrucosum 5 T. equinum)

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