Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2012, 3 (4):2073-2077

ISSN: 0976-8610
CODEN (USA): AASRFC

Screening of fungi isolated from poultry farm soil for keratinolytic activity

Mini K. D*, Mini. K. Paul and Jyothis Mathew

Sree Sankara College, Kalady. School of Biosciences, Kottayam

---

ABSTRACT

Poultry farm soil samples collected from different localities of Ernakulum and Thrissur districts of Kerala were screened for the keratinolytic fungi. During the course of study 8 different fungi were isolated and identified. Aspergillus, Chrysosporium, Microsporum, Trychophyton and Penicillium were the fungi isolated and were grown in wheat bran substrate. Feather keratin powder was added to the substrate to enhance the enzyme production. They were found utilizing keratin substrate releasing keratinase enzyme into the medium. These enzymes were assayed for their activity. Some cultural conditions were tested to attain maximum Keratinase production. Maximum enzyme production was reached on the 4th day of incubation of the culture at 37^{o}C and pH 8.5

Key words: kerainolytic fungus, keratin, keratinase

INTRODUCTION

Fungi are an important component of the soil microbiota more in abundance than bacteria; their population depends upon soil depth and nutrient conditions. The soil samples were collected from different sites of Ernakulam and Trichur districts. The soil collection mainly focussed the proximities of poultry farms, dumping sites of animal hair, hoof, nail etc. Different soils have specific fungus flora, but the majority of species found in them are cosmopolitan, (Ainsworth & Sussman, 1968). Fungi present in the soil include keratinophilic (keratin loving) and some keratinolytic (keratin digesting) strains. Many of them are potential pathogen to both human and animals. Soils that are rich in keratinous materials are most conducive for the growth and occurrence of keratinophilic fungi (Moallaei & Zaini, 2006). Keratin is a major component of feathers. Among the microbes that cycle keratin protein in nature, keratinophilic fungi are very common and the most diverse. If keratinolytic fungi were not there to cycle this highly stable protein (keratin), we can imagine the quantity of keratin that would have accumulated on earth, since a vast quantity of keratin is shed by the vertebrates. Indian soils contain many more keratinophilic fungi than those presently recorded, and there is need for further taxonomic and ecological studies of this interesting group of organisms (Sharma R & Rajak RC (2003). In general, the qualitative and quantitative composition of keratinolytic fungi can be a multifunctional bioindicator of environmental pollution with waste. It means that the composition indicates not only the presence of keratin remnants and faecal contaminants in the environment but also respond to the changes in environmental conditions. (K Ulfig 2000) Fermentation using feather as a substrate was carried out on minimal salt media for 7 days which resulted in almost complete degradation of feather. (Avinash Srivasthava *et al.*, 2011) The amount keratinase production depended on substrate concentration and cultivation conditions. In the present study highest keratinase production was observed on substitution with 2% feather meal. The potential use of keratinases have different applications where keratins should be hydrolysed, such as the leather and detergent industries, textiles, waste bioconversion, medicine, and cosmetics for drug delivery through nails and degradation of keratinized skin. Fungi also display lipolytic activity and remove petroleum hydrocarbons from the medium during degradation of proteins.

A distinctive feature of keratin is its relatively high sulphur content due to the presence of sulphur containing amino acids viz. cystiene, cysteine and methionine. Thus, the disulfide bonds are considered to be responsible for the stability of keratin and its resistance to enzymatic degradation (Kunert, 1989). Keratinolytic mycoflora love to grow

and even reproduce on keratin materials such as skin, hair, nail, fur, feather, horn, hoof, beak etc. They utilize keratin as carbon source (Cooke, 1980). Keratinophilic fungi are important ecologically and present in the environment with variable distribution patterns and cause human and animal mycoses (Mohamed *et al*., 2000). Keratiophilic fungi display potentially pathogenic properties to animals, including human beings. Studies of these fungi in the environment are therefore of hygienic and epidemiological importance. (Avasn *et al.*, 20121) The risk of fungal infections is increasing in the environment contaminated with sewage. The results however indicated that the use of Hair Bait method and dilution techniques facilitated the recognition of keratinophilic mycoflora.((Avasn *et al.*, 2012[2]) This study reports the prevalence of keratinolytic fungi from poultry farm soil collected from different locations of Ernakulam and Trichur districts in Kerala.

MATERIALS AND METHODS

Sterilised feathers, keratin, keratin salt medium, soil, distilled water, petridishes and Sabouraud dextrose agar (SDA), Lactophenol cotton blue etc. were needed for the study.

Collection of Soil: Soil was collected in sealed containers using sterile spoon from the poultry farm premises where keratin and hence keratinolytic fungi were present.

Isolation of Keratinolytic Fungus

10gm of the soil was mixed with 100ml. of distilled water and the dilutions were plated on keratin agar medium (gm/250ml) containing keratin-2.5, $MgSO_4$ - 0.25, KH_2PO_4 – 0.115, K_2HPO_4 - 0.25 and Agar – 5. Streptomycin 1% was mixed with the medium. Plates were incubated at 37^{o}C for 5 days. The plates in which clear zones were seen indicated the fungi with keratinolytic activity. They were carefully isolated and stored in SDA (Saboraud Dextrose agar) medium (g/250ml) Peptone – 2.5, Dextrose – 10 and Agar – 5.

Identification of Fungus

The isolated fungi were stained with Lactophenol cotton blue and observed it under the microscope. By noting the morphology of the fungus identification was done. For the selection of the fungi with high keratinolytic activity, already identified fungi were cultured in feather keratin substrate.

Preparation of feather keratin substrate.

Fairly large amount of chicken feather was collected from the poultry farm and washed well with chloroform-methanol (1:1, v/v), and finally with distilled water. It was then dried in sunlight and sized into 1cm length. Sterilization was done by tyndallization at 100˚C for 20 min on five successive days. It was then powdered in sterile condition.

Preparation of Medium for fungal culture

The medium was prepared for inoculating the fungus was as follows (g/l): wheat bran - 10, KH_2PO_4 - 0.46, K_2HPO_4 - 1, $MgSO_4$-0.5, at a pH 8.5. Erlenmeyer flasks containing 20 m*l* of sterilized medium supplemented with 10gm of sterilized feather as a keratin source was incubated at 37˚C and 120 rpm for 4-5 days. Flasks containing the medium with a disc of agar without the fungus served as control. For each species, one test flask and one control set were maintained. For inoculation, spore suspensions $2x10^{8\ of}$ 5 day-old inoculum was used.

Extraction of Enzyme

Phosphate buffer having pH 8.5 was used for the extraction of culture filtrate. After 4-5 days of incubation, 100ml. of PO_4 buffer was added to each flask. The flasks were rotated in the shaking incubator at 200rpm for 1 hr. at 37^{o}C. After 1 hr. the ingredients were filtered through whatman's No: 1 filter paper and the filtrate were used for enzyme activity.

Enzyme activity.

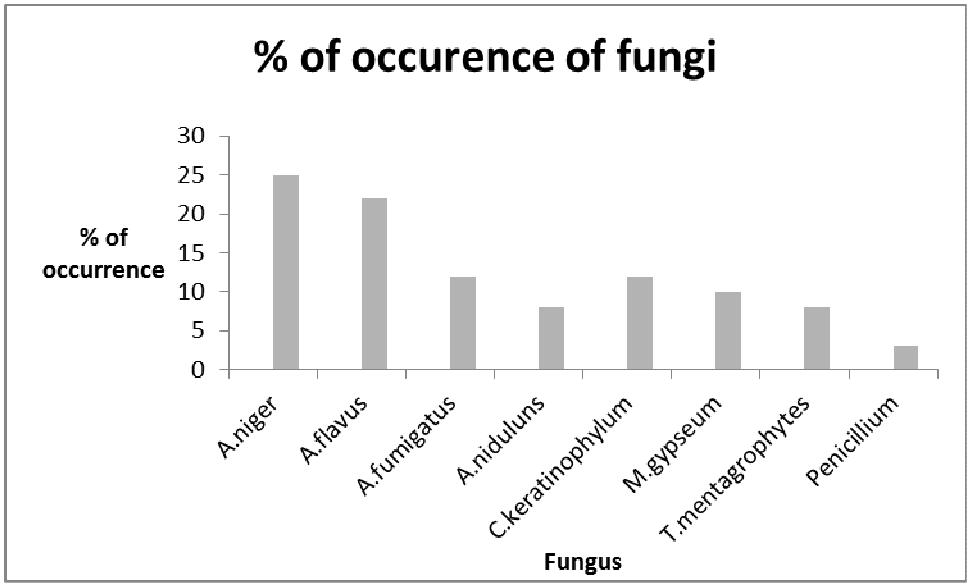
Keratinase was assayed by Lowry *et al* method. One unit of enzyme activity is defined as the amount of enzyme liberating one microgram of tyrosine per minute per ml. under the defined conditions. The activity of the enzyme is expressed in units/gds (gram dry weight)

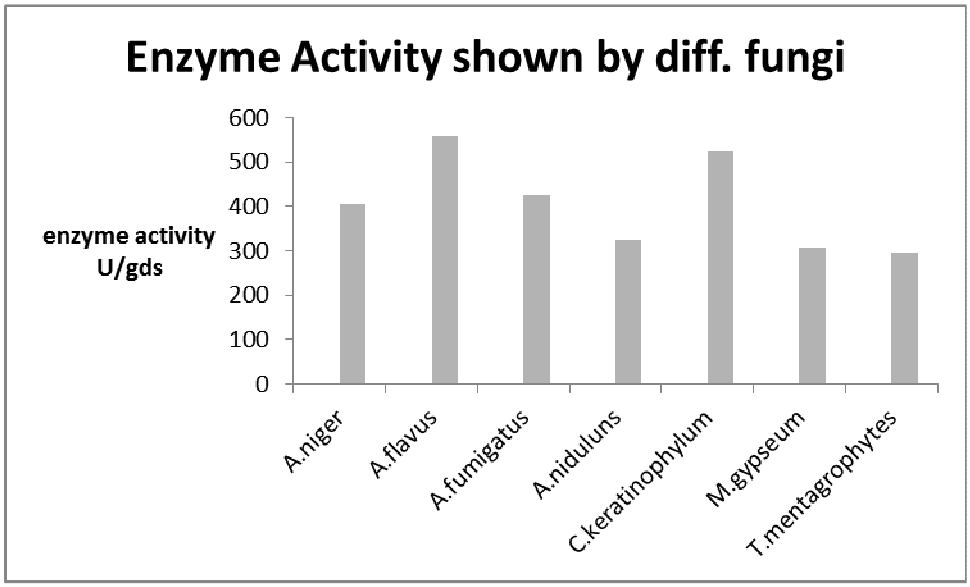
RESULTS AND DISCUSSION

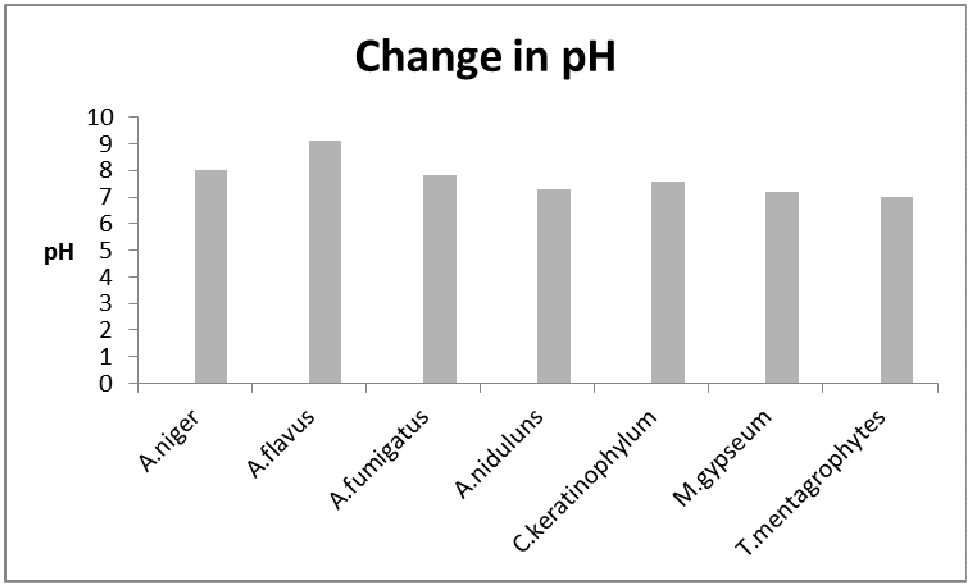
Out of 60 soil samples collected from the poultry farm premises, 51 samples were found positive in fungal growth. A total of 5 genera and 8 species were isolated. In the study some of the soil samples gave single species and some yielded mixed growth of two or more species of fungi. Aspergillus species was isolated frequently. The following table reveals the % occurrence of fungi isolated from the soil.

Fungi isolated & % of occurrence

Fungus	% of Occurence	Enzyme ActivityU/gds
Aspergillums Niger	25	406
A.flavus	22	560
A.fumigatus	12	425
A.nidulans	8	324
Chrysosporium keratinophylum	12	523
Microsporum gypseum	10	308
Trychophyton mentagrophytes	8	297
Penicillium sps.	3	







Among the seven species of fungi better activity was recorded in A.flavus (560U/gds) followed by Chrysosporium keratinophylum (523U/gds) A.fumigatus (425U/gds), A.niger (406U/gds) A.niduluns (324U/gds) Microsporum gypseum (308U/gds) and Trychophyton mentagrophytes (297U/gds) The extracellular enzymes secreted by these fungi are responsible for the degradation of keratin in nature. Change in alkalinity may be due to the high alkaline nature of the medium due to cysteine, keratinase and protein. Evidences of keratinolysis lie on the ability of fungi to release soluble sulphur containing aminoacid and polypeptides into the medium. Santos *et al*. (1996) have investigated that *A.fumigatus* was useful for the microbial conversion of keratinous waste and *A. flavus* has been selected as a prospective producer of a keratinolytic enzyme (Gradišar *et al*., 2000)

CONCLUSION

Large number of microbes is present in the environment which is capable of recycling keratin protein. Among them keratinolytic fungi are most diverse and so common. The capacity of these fungi can be exploited for degrading the keratinous wastes and thus an effective means of bioremediation.

REFERENCES

[1] G.C Ainsworth, A.S Sussman, *The Fungi an Advanced Treatise* Vol. III *the Fungal Population*, **1968**, pp. 426-496
[2] Y. Avasn Maruthi, D.Apta Chaithanya, Kaizar Hossain, A. Sravani , S.Jagadish , *European Journal of Experimental Biology*, **2012**, 2(1) : 13-16
[3] Y. Avasn Maruthi , D. Kaizar Hossain, Hari Priya and B. Tejaswi , *Advances in Applied Science Research*, **2012**, *3 (1):605-610*
[4] Avinash Srivasthava , Anshul Sharma, Vippu Suneetha, *European journal of Experimental biology*, **2011**, 1(2): 56-63
[5] P. Abu P, A. Hilda , and S.C. Gopinath , *Mycopathologia*, **2004**, 158(3): 303-909.
[6] G.P.Bodey , S.Vartivarian , *Eur. J. Clin. Microbiol. Infect. Dis*,**1989**, 8: 413-437.
[7] R.C.Cooke , Fungi *Man and Environment.* Longman Group Ltd. London **1980**, pp. 17-19.
[8]M.V. Fillipello , *Keratinophilic fungi: Their role in nature and degradation of keratinic substrates. In: Biology of dermatophytes and other keratinophilic fungi, Kushawaha* RKS, Guarro J, editors. Revista Iberoamericana de Micologia. Spain, **2000**, pp. 77-85.
[9] H. Gradišar , S.Kern , J. Friedrich , *Appl. Microbiol. Biotechnol*.**2000**, 53: 196-200.
[10] Z. Jesenka , J. Durkovsky , I. Rosinsky , M. Polak , E. Zamboova , B. Baca *Mikrobol. Imunol*, **1992** , 41: 337-341.
[11] Krzyszto Ulfig, *Journal of Agricultural and Biological Research*, **2000**, 1(2) : 44
[12] J. Kunert , Keratin *decomposition by dermatophytes. I.Sulfite production as a possible way of substrate denaturation.Z. Allg. Mikrobiol*, **1973**, **13**: 489-498.
[13] H.Moallaei , F. Zaini , *Iranian J. Publ. Health*, **2006**, 35(4): 62-69.
[14]M.D Richardson, M. Kokki , *Aspergillus*. In: *Clinical Mycology*. (Eds.): **2003**, E. Anaissie, M.McGinnis, *M. Pfaller ChrchillLivingstone* New York.

[15] Samuel Pandian1, Jawahar Sundaram1 and Prabakaran Panchatcharam, *European Journal of Experimenal Biology*, **2012**, 2(1): 274-282
[16]R. Santos,M.D.B, A.A.P Fermion , C.M. de Sá, C.R.Felix,1996. *Curr. Microbiol*. **1996**, 33: 364-370.
[17] M.I.M. Sarquis ,P.C. Oliveira , *J. Basic Microbiol*, **1990**, 36: 51-58.
[18] R.Sharma, R.C.Rajak, *Resonance*, **2003** pp. 28-40.