

Degradation of few avean feathers by *Microsporium gypseum*

N.C.Sowjanya*¹ and C.Manohara Chary²

*¹Govt.City College (A), Hyderabad, Andhra Pradesh, India.

²Department of Botany, University College of Science, Osmania University, Hyderabad 500007, India.

Abstract

The ability of the keratinophilic fungus *Microsporium gypseum* to degrade different keratin substrates viz., chicken feathers, pigeon feathers and peacock feathers has been studied under different incubation periods. The amount of net protein ($\mu\text{g/ml}$) released during the growth of *Microsporium gypseum* on different keratin substrate reveals that pigeon feathers are most degraded keratin substrate and peacock feathers are least degraded keratin substrate.

Keywords: Chicken feathers, *Microsporium gypseum*, peacock feathers, pigeon feathers

INTRODUCTION

Keratins are proteins with extremely high molecular weight. They are resistant to digestion by pepsin and trypsin, insoluble in dilute acids, alkalis, water and organic solvents. They have high sulphur content amino acid in the form of cystine. The resistance to solvents and enzymes is due to close packing of these chains. Fungi capable of colonizing natural keratin such as skin, feathers, hair, horn, hoof etc are widespread in nature and probably fulfill a vital function in the breakdown of hard keratin detritus of man and animals to simple organic compounds [1, 2]. These fungi are known to have a specialized enzymatic system which enables them to break down the keratin, a complex protein to simple organic compounds. In the present study an attempt has been made to study the ability of a keratinophilic fungus, *Microsporium gypseum* to degrade pigeon feathers, peacock feathers and chicken feathers. The addition of keratin substrates stimulated the growth of keratinophilic fungi. They deteriorate them very rapidly and release high amount of protein [3-6]. It is now well established that the breakdown of keratin is carried out by the action of extracellular enzymes, keratinases [7-12].

MATERIALS AND METHODS

The keratin substrates used in the present study are peacock feathers, chicken feathers and pigeon feathers.

Preparation of the keratin substrates and the inoculum

The keratin substrates were sterilized with a mixture of chloroform-methanol (1:1: v/v) renewed several times in 24 hours, washed twice with glass distilled water and air dried. Mineral medium containing 1.5g of K_2HPO_4 , 0.25g MgSO_4 , 0.005g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.025g CaCl_2 , 0.005g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 30g Dextrose per litre of distilled

water (pH 6.5) was used in all the experiments. Inoculum was the conidial suspension from the surface of 6 days old single spore cultures. The conidial suspension was obtained from culture tubes by brushing conidia in 5ml of sterilized distilled water and 2ml of conidial suspension (300 conidia per ml) was added to each flask containing basal liquid medium. Each 100ml Erlenmeyer flask received 250mg of the sample. The cultures were incubated in stationary condition at $28 \pm 2^\circ\text{C}$. The following are the treatments.

1. Keratin control to which was added 30 ml of mineral medium and 250 mg of the keratin substrate.
2. Fungus control to which was added 30 ml of mineral medium and 2 ml of fungal inoculum.
3. Test samples to which were added 30 ml of mineral medium, 250 mg of keratin substrate and 2 ml of fungal inoculum.

Substrate decomposition and determination of soluble proteins

The protein determinations from filtrates were carried out from the flasks of all the three experimental sets after different periods of incubation. The filtrate from each flask was centrifuged at 4,000 rpm for 5 minutes and the supernatant was assayed for protein by using Folin ciocalteu reagent as described by Lowry *et al.*[13] and Packet [14]. The developing colour was read at 660 nm on spectrophotometer. BSA was used as the standard. The results of protein estimation were expressed as net values i.e., the measured value in the test sample minus the sum of values of keratin and fungus controls. All the experiments were carried out in triplicate.



Fig 1.

Received: Feb 11, 2012; Revised: March 22, 2012; Accepted: April 25, 2012.

*Corresponding Author

N.C.Sowjanya
Govt.City College (A), Hyderabad, Andhra Pradesh, India.

Tel: +91-9490119977
Email: jaisahithi@yahoo.com

RESULTS

In the present investigation the ability of the keratinophilic fungus *Microsporium gypseum* to degrade different keratin substrates – chicken feathers, pigeon feathers and peacock feathers (Fig.1) has been studied under different incubation periods (day-wise, weekly).The net protein released by the *Microsporium gypseum* during the degradation of different keratin substrates under different incubation periods (day-wise and weekly) are tabulated in the Table – 1 and Table – 2 respectively.

Observation of degradation of Chicken feathers: (day-wise and weekly)

The perusal of the Table – 1 gives a picture of the amount of protein released from the chicken feather during the growth of *Microsporium gypseum* (day-wise).A linear increase in the amount of protein released was observed.The lowest (252 µg/ml) being recorded on 2nd day and highest (339 µg/ml) on 10th day. The values are presented in terms of standard error [Mean ± S.E (n=3)]. The results furnished in the Table – 2 depicts the amount of protein released from the chicken feathers during the growth of *Microsporium gypseum* (weekly). It is evident from the results that there is a steady increase in the amount of protein release from 1st week to 4th week. Minimum (298 µg/ml) was recorded in the first week and maximum (336 µg/ml) was recorded in the 4th week. The data is expressed in terms of standard error [Mean ± S.E (n=3)].

Observation of degradation of pigeon feathers :(day-wise and weekly)

Table – 1 gives a picture of the amount of protein released from pigeon feathers during the growth of *Microsporium gypseum* (day-wise). It is evident from the values given in the table that there is a gradual increase in the amount of protein release from 2nd to 10th day. Minimum amount (430 µg/ml) was recorded on 2nd day and maximum (506 µg/ml) was recorded on 10th day. The data is expressed in terms of standard error [Mean ± S.E (n=3)]. The results presented in the Table – 2 summarizes the amount of protein released from the pigeon feathers during the growth of *Microsporium gypseum* (weekly). It is evident from the data that the amount of protein released increased gradually from 1st week to 4th week. Minimum (482 µg/ml) was recorded in the 1st week. Maximum (614 µg/ml) was recorded in the 4th week. Statistically analyzed data is expressed in terms of standard error [Mean ± S.E (n=3)].

Observation of degradation of Peacock feathers :(day-wise and weekly)

The results presented in the Table –1 summarizes the amount of protein released from peacock feathers during the growth of *Microsporium gypseum* (day-wise). Varying results were obtained. The amount of protein released increased by 4th day followed by a decrease on 6th and 8th days. The observations of the result of 10th day revealed a slight increase in the amount of protein released over 8th day. Statistically analyzed data is expressed in terms of standard error [Mean ± S.E (n=3)].The perusal of the Table – 2 reveals the amount of protein released from the peacock feathers during the growth of *Microsporium gypseum* (weekly). The result of the 1st, 2nd and 3rd weeks reveal that there is an increase in the amount of

protein release with the increase in the incubation period. However, the result of the 4th week depict that the protein release decreased sharply from that observed in the 3rd week. The data is presented in terms of standard error [Mean ± S.E (n=3)].A Comparative study on the amount of net protein (µg/ml) released during the growth of *Microsporium gypseum* on different keratin substrate reveals that pigeon feathers are most degraded keratin substrate and peacock feathers are least degraded.

Table 1. Net protein (µg/ml)* released during the growth of *Microsporium gypseum* on different keratin substrates (Day-wise)

Incubation period (days)	Chicken feathers	Pigeon feathers	Peacock feathers
2	252	430	178
4	284	499	303
6	295	481	207
8	302	489	149
10	339	506	213

*Net protein released = Test sample – Sum of keratin control and fungus control

Table 2. Net protein (µg/ml)* released during the growth of *Microsporium gypseum* on different keratin substrates (Weekly)

Incubation period (weeks)	Chicken feathers	Pigeon feathers	Peacock feathers
1	298	482	162
2	315	495	301
3	320	509	437
4	336	614	168

*Net protein released = Test sample – Sum of keratin control and fungus control

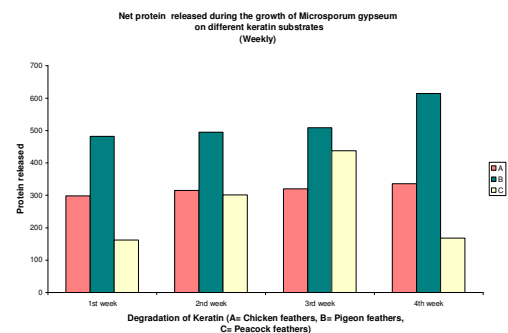


Fig 2.

DISCUSSION

In the present investigation the ability of *Microsporium gypseum* to degrade chicken feathers, pigeon feathers and peacock feathers has been studied from 2nd day to 10th day and also from 1st week to 4th week. Net protein released has been estimated. The degradation of pigeon feathers was more rapid than other substrates and the least degraded keratin substrate is the pigeon feather. The type of feather composition of feathers, physical nature of the feather, the efficacy of the fungal isolate and also the incubation period are the factors responsible for different rates of keratin degradation. Kunert [15] has reported rapid digestion of human hair by his isolate of *Microsporium gypseum*. Nigam and Kushwaha [5-6] have also reported the degradation of keratin substrates by *Microsporium gypseum*. Parihar and Kushwaha [6] have also reported that *Microsporium gypseum* could degrade hen feathers more rapidly than other keratin substrates. After the isolation and purification of extracellular keratinase of *Trichophyton mentagrophytes*, by Yu et al.[16] was proved that these dermatophytes and related

keratinophilic fungi possess a specific enzyme enabling its parasitic growth in the keratinized layers of skin, nails, hair, horns etc. Hence, intensive studies on keratinases of these fungi will certainly contribute towards better understanding of keratin degradation.

REFERENCES

- [1] Ajello, L.1953. The dermatophyte, *Microsporum gypseum*, as a saprophyte and parasite. *J.invest.Dermatol.*22: 17-21.
- [2] Gordon, M.A. 1953. The occurrence of the dermatophyte *Microsporum gypseum* as a saprophyte in soil. *J.Invest.Derm.*20: 201-206.
- [3] Kushwaha, R.K.S. 1983. The *in vitro* degradation of peacock feathers by some fungi. *Mykosen.*26: 324-326.
- [4] Kushwaha, R.K.S. 1995. Biodeterioration of feathers by Keratinomycetes isolated from museum of Spain. 3rd *International Conference on Biodeterioration of Cultural Property.* pp.298-302.
- [5] Nigam, N.and R.K.S.Kushwaha.1989. Decomposition of feathers and hairs by Keratinophilic fungi. *Indian J.Microbiol.*29: 241-244.
- [6] Parihar, P.and R.K.S.Kushwaha.1999. Decomposition of feathers by some keratinophilic fungi.*J.Mycol.Plant Pathol.*29: 192-196.
- [7] Biswas, S.B., M.Gupta and G.R.Ghosh.1988. Keratinase activity in some gymnoascaceous fungi. *Kavaka.*14: 81-85.
- [8] Das, S.K.and A.B.Banerjee.1982. Effect of Undecanoic acid on the production of exocellular lipolytic and keratinolytic enzymes by Undecanoic acid sensitive and resistant strains of *Trichophyton rubrum*. *Sabouraudia.*20:179-184.
- [9] El-Naghy, M.A., M.S.El-Ktatny, E.M.Fadl-allah and W.W.Nazeer.1998. Degradation of chicken feathers by *Chrysosporium georgiae*. *Mycopathologia.* 143: 77-84.
- [10] Hasija, S.K., H.Malviya and R.C.Rajak.1990. Keratinolytic ability of some fungi isolated from gelatin factory campus Jabalpur, M.P.*Proc.Nat.Acad.Sci.India.*Part III Section B.pp.305-309.
- [11] Higuchi, D., I.Takiuchi and M.Negi.1981. The effect of keratinase on human epidermis especially on statum corneum. *Jap. J. Derm.*91: 119-125.
- [12] Malviya, H.K., S.Parwekar, R.C.Rajak and S.C.Hasija.1992. Evaluation of Keratinolytic potential of some fungal isolates from gelatin factory campus. *Indian J.Exper.Biol.* 30: 103-106.
- [13] Lowry, O.H., N.J.Rosebrough, A.L.Farr and R.J.Randall.1951. Protein measurements with folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- [14] Packer, L.1967. *Experiments in cell physiology*, Academic Press, London. pp.134-135.
- [15] Kunert, J. 1972. The digestion of human hair by the dermatophyte *Microsporum gypseum* in a submerged culture. *Mykosen.* 15: 59-71.
- [16] Yu, R.J., S.R.Harmon and F.Blank.1968. Isolation and purification of an extracellular keratinase of *Trichophyton mentagrophytes*. *Journal of Bacteriology.* 96: 1435-1436.