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Determination of Keratinolytic Potential of Keratinolytic Fungi on Poultry Feather Samples Isolated from Selected Poultry Sites around Shivamogga, Karnataka, India.



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ABSTRACT

The keratinolytic potential of the keratinolytic fungi was determined by the release of protein by utilizing feather keratin. The total protein yield was found to be 430 ug mL⁻¹ from *Chrysosporium indicum*, 410 ug mL⁻¹ protein from *Microsporum canis*, 390 ug mL⁻¹ protein from *Chrysosporium keratinophilum*, 370 ug mL⁻¹ protein from *Microsporum gypseum*, 360 ug mL⁻¹ protein from *Trichophyton rubrum*, 355 ug mL⁻¹ protein from *Epidermophyton floccosum*, 350 ug mL⁻¹ protein from *Chrysosporium lobatum*, 345 ug mL⁻¹ protein from *Microsporum nanum*, 335 ug mL⁻¹ protein from *Chrysosporium tropicum*, 320 ug mL⁻¹ protein from *Trichophyton terrestre*, 300 ug mL⁻¹ each by *Trichophyton mentagrophytes* and *Chrysosporium annum* and 250 ug mL⁻¹ protein from *Trichophyton ajelloi* after 60 days of incubation with sterile feather samples. By the results we concluded that, out of all tested keratinolytic fungi, *Chrysosporium indicum* most effective keratinolytic fungi in degrading feather samples.



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INTRODUCTION

Keratins are the waste products of the animal and have the role in protecting body form unfavorable climatic conditions. Keratin is scleroprotein group, has numerous cross-links of disulfide bonds which is hard to degrade and chemically inert, so only few organisms able to make it as energy source. Keratin substances include hairs, horns, feathers, nails etc. (Asquith, 1977; Timar-Balazsy and Eastop, 1998).

Majchrowicz & Dominik (1969) differentiate keratinolytic fungi from keratinophilic fungi; keratinolytic fungi are able to attack and completely degrade keratin, whereas keratinophilic fungi associates keratinolytic fungi, utilizing only non-keratinous components of keratinous substrata or the products of keratin decomposition.

Poultry feather founds the most abundant keratinous material in nature. In mature chickens total body weight, feathers represents 5-7% and in commercial poultry processing plants, every year more than 20,000 tons of feathers are produced as waste (Vogt and Stute, 1975). Around 8.5 billion tons of poultry feather is generated annually from poultry processing plants worldwide, India's contribution is 350 million tons. Due to lack of knowledge in developing countries, poultry wastes are unscientifically dumped/buried in the soil, which pollute the soil or burnt in field which pollute the air (Saha S. 2013).

Poultry feather looks like great threat to the environment but, understandably, they are potentially useful protein /amino acids that their digested product could be used as animal feedstuff. This thinking may helpful in recycling of feather and in other hand prevent the soil and air pollution. But, even though it is a cheap and alternative protein feedstuff it cannot be successfully commercialized due to lack of standard procedures (Onifade et al 1998).

Keratinolytic fungi are ecologically an important group of fungi which will feed on the very tough material called keratin, abundantly found in soil called Geophilic dermatophytes, some groups of these fungi are causative agents of cutaneous fungal infections named dermatophytosis and the other saprophyte fungi mainly represent hyalohyphomycosis (Deshmukh, et al., 2006 and Pålsson, 1968).

Environmental factors play an important role in the growth and sporulation of keratinolytic fungi. Fungi grow best at optimum temperature and related humidity. Both the factors govern metabolic activities of growing organism. The extremely high and very low temperature decreases the growth of keratinolytic fungi. The increased level of relative humidity shows excellent growth (Sharma, et al., 2012).

The distribution of Geophilic dermatophytes and other keratinolytic fungi individual populations was not uniform in all the places, they depends on the environmental and edaphic factors viz., Energy resource (keratin), Soil pH, Humidity, Temperature, Geographical location and also Density of human population (Kowalska, et al., 2011).

There is no standardized procedure on the prospects for industrial applications of keratinolytic microorganisms, especially with importance on the degradation of keratin, therefore, Considering the importance in the keratin waste management, this study aimed at the keratinolytic potential of few keratinolytic fungi isolated from poultry farm of Shivamogga, Karnataka.

MATERIALS AND METHODS

Collection of poultry samples

A total of five soil samples were collected randomly from different poultry farms located nearby Shivamogga City, Karnataka state, (India) over a period of four months from July to October 2014 in polythene bags and transported to laboratory for further analysis.

Isolation of keratinolytic Fungal isolates

The hair bait technique suggested by Vanbreuseghem, (1952) was used to isolate the keratinolytic fungi. Sterile petri dishes were half-filled with poultry soil samples and half filled with sterile poultry feather samples and moistened with water periodically. These dishes were incubated at room temperature five days and examined daily for fungal growth until four weeks. When the fungal growth was observed, it was cultured on Sabouraud's dextrose agar medium supplemented with Chloramphenicol (50 mg L^{-1}) and cycloheximide (500 mg L^{-1}).

Identification and characterization of keratinolytic Fungal isolates

The isolated fungi were identified on the basis of spore morphology, cultural characteristic and pigment formation on the reverse of slant (Forbes et al., 2002) and individual identification of keratinolytic fungi was microscopically done using standard keys and monographs suggested by Rippon, (1988); Rebell, (1974); Frey et al., (1979); Van Oorshot, (1980); cano J Gurrao, (1990).

Determination of Keratinolytic Potentials of Fungal Isolates

Feather samples were washed in solution of detergent and rinsed in distilled water. These were sterilized at 121°C for 15 min. The sterile hairs were defatted in mixture of chloroform and methanol (1: 1) and washed twice with sterile distilled water. The air-dried feathers were cut into pieces and 1 g weighed cut feathers were inoculated with ten days old mycelial culture on Sabouraud's dextrose agar medium and incubated at room temperature for 40-60 days. Water is sprinkled whenever necessary. After 60 days the digested feather samples were immersed in double distilled water for 1 hour and filtered, undigested debris were discarded and water is collected. The total protein was estimated according to the method described by Lowry et al. (1951) by using Bovine serum albumin as a standard and Folin-Ciocalteu as reagent. The controls were without inoculum or substrate used to compare the results.

RESULTS AND DISCUSSION

Identification and characterization of keratinolytic Fungi

Individual fungi were identified on the basis of spore morphology, cultural characteristic and pigment formation on the reverse of slant (Forbes et al., 2002) and identification of keratinolytic fungi was microscopically done using standard keys and monographs suggested by Rippon, (1988); Rebell, (1974); Frey et al., (1979); Van Oorshot, (1980); cano J Guarro, (1990), 13 species keratinolytic fungi which were successfully isolated and identified belongs to 4 genera viz., *Chrysosporium*, *Microsporium*, *Epidermophyton*, *Trichophyton*.

Keratinolytic potential of isolated keratinolytic fungi

Sixty days incubated feather sample filtrate were subjected total protein estimation method described by Lowry et al 1951. A standard graph is plotted with concentration of protein on X

axis and absorbance on Y axis (Chart 1) revealed that out of all experimented keratinolytic fungi *Chrysosporium indicum* was the effective keratin degrading fungi in our climatic region which give 430 ug mL^{-1} total protein from 1 gram of sterile feather sample, it may be due to the quick adaptation to the Indian climatic conditions.

Previous Indian researchers (Garg AK. 1966) were revealed the quick adaptation of *Chrysosporium indicum* in Indian climatic conditions.

Next effective keratin degrading fungi is *Microsporum canis* gives 410 ug mL^{-1} of protein from 1 gram of feather sample and *Chrysosporium keratinophilum* which yields 390 ug mL^{-1} of total protein in third position (Chart 2).

Other keratinolytic fungi like *Microsporum gypseum* (370 ug mL^{-1}), *Trichophyton rubrum* (360 ug mL^{-1}), *Epidermophyton floccosum* (355 ug mL^{-1}), *Chrysosporium lobatum* (350 ug mL^{-1}), *Microsporum nanum* (345 ug mL^{-1}), *Chrysosporium tropicum* (335 ug mL^{-1}), *Trichophyton terrestre* (320 ug mL^{-1}) shows moderate keratin degrading activity.

Trichophyton mentagrophytes and *Chrysosporium annuum* gives 300 ug mL^{-1} each from 1 gram of feather sample.

Trichophyton ajelloi was the least protein yielding fungi which gives 250 ug mL^{-1} total protein from 1 gram of feather sample.

It is a modified experiment of the Tumbekar D H et al 2007 because usually some keratinolytic fungi didn't grow in basal salt solution so that, for accurate results we inoculate 10 days old pure culture directly to the sterile feather sample instead of feather sample in basal salt medium.

Similar experiments previously conducted by other scientist got somewhat comparable results for *Microsporum canis*. But, for few keratinolytic fungi's like *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum gypseum*, we got more protein yield which was less in their results and *Microsporum gypseum* yield less protein which was effective protein degrading fungi from Tumbekar D H et al 2007 work. This variation of the results from previous experiments was may be due to the influence of external climatic factors.

Table 1: Protein yield by keratinophilic fungi

Sl no	Fungal isolates	Absorbance at 750 nm	Protein yield ($\mu\text{g mL}^{-1}$)
1	<i>Trichophyton ajelloi</i>	0.5	250
2	<i>Chrysosporium annum</i>	0.6	300
3	<i>Trichophyton mentagrophytes</i>	0.6	300
4	<i>Trichophyton terrestre</i>	0.64	320
5	<i>Chrysosporium tropicum</i>	0.67	335
6	<i>Microsporum nanum</i>	0.69	345
7	<i>Chrysosporium lobatum</i>	0.7	350
8	<i>Epidermophyton floccosum</i>	0.71	355
9	<i>Trichophyton rubrum</i>	0.72	360
10	<i>Microsporum gypseum</i>	0.74	370
11	<i>Chrysosporium keratinophilum</i>	0.78	390
12	<i>Microsporum canis</i>	0.82	410
13	<i>Chrysosporium indicum</i>	0.86	430

Chart 1: Standard graph by plotting absorbance against the concentration

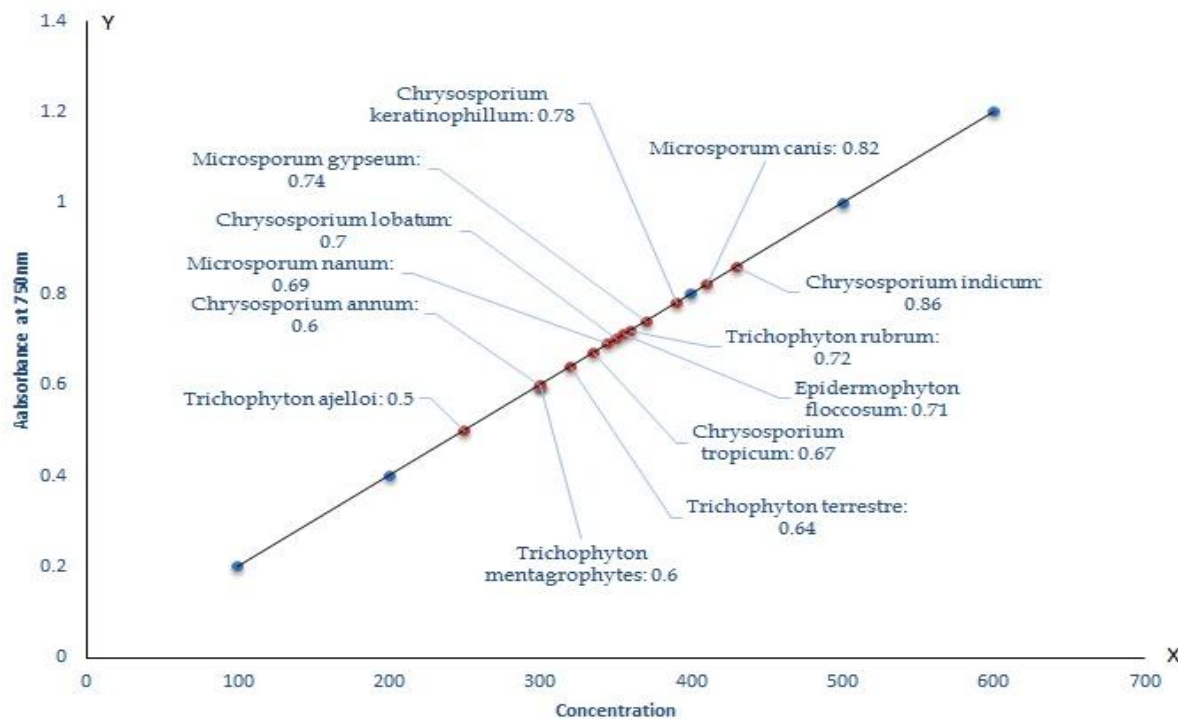
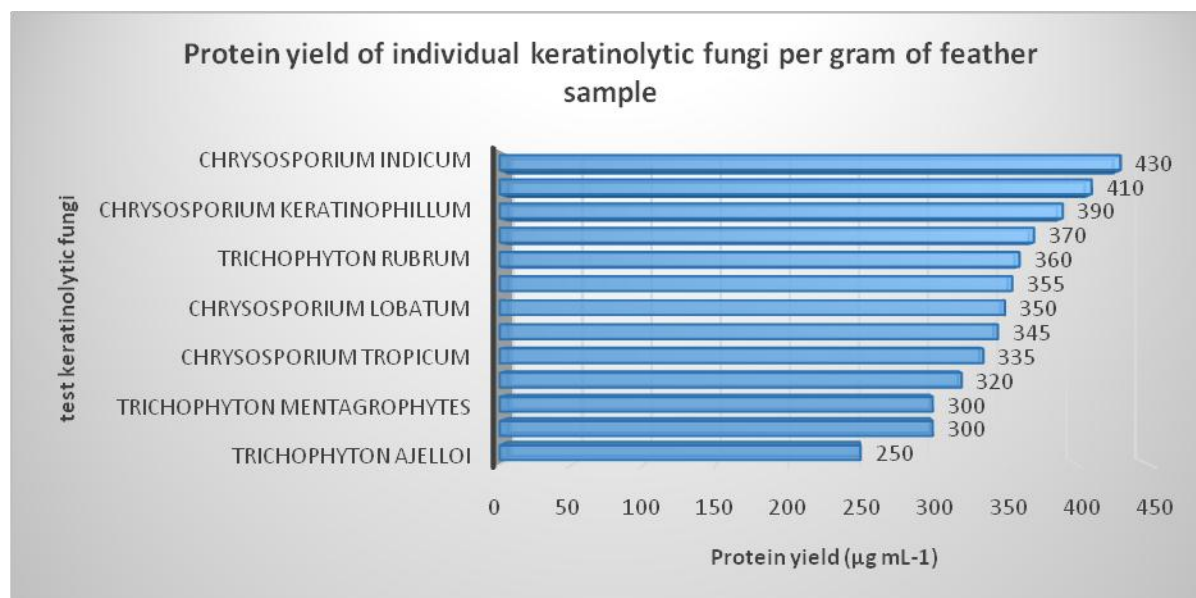


Chart 2: Protein yield of the individual keratinolytic fungi per gram of feather sample



CONCLUSION

From our results, it is concluded that *Chrysosporium indicum* was the effective keratin degrading fungi from our climatic region which give 430 $\mu\text{g mL}^{-1}$ total protein from 1 gram of sterile feather sample and rest of the keratinolytic fungi shows appreciated keratinolytic activity. The variation in keratinolytic activity of tested fungi may be the influence of climatic factor of that particular place where experiment was conducted. *Chrysosporium indicum* shows appreciated keratinolytic activity may be due to the quick adaptation to climatic conditions prevailing in this area.

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