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Impact Of Temperature And Ph On Degradation Of Poultry Feather Wastes Throuh Keratinolytic Fungi

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Abstract

Feathers are produced in large amount as a waste by- product from poultry industry. Millions of tons of feathers are produced annually worldwide. Keratinolytic fungi have a great importance in poultry waste degradation and bioconversion to fertilizers, feedstuffs. The aim of this study was to focus on the feather degrading ability of *C. keratinophilum* and *M. gypseum* in different temperature and pH. Various temperature regimes of 18^0c, 28^0c, 38^0c, 42^0c were used to determine the temperature effect on feather weight loss. These fungi showed their maximum keratinolytic ability at 28 ^{0}c. Maximum weight loss was obtained at 28^0c. These fungi were also cultured on 4, 5, 6, 7, 8, 9 pH regimes. The keratin degrading ability was found somewhat better towards alkalinity than the acidic condition. The great ability of selected keratinolytic fungi to degrade feathers can be utilized for their potential biotechnological application in processing of feather waste from poultry industry.

KEYWORDS: Keratinolytic fungi, feather, keratin, poultry waste, keratinolytic ability.

Introduction

Keratinophilic fungi that decompose keratinous substrate have got attention in recent days and are an important component of the soil microbiome. Physical and chemical factors play an important role in growth and sporulation of keratinophilic fungi and dermatophytes. Keratinophilic fungi can be considered as nature's keratin degrading machine.[1]

India is the major contributor in production of keratinous waste material. Feather which accounts for 5-8% of the total weight of mature chickens are produced in large amount as a waste by – product from poultry industry. Millions of tons of feathers are produced annually worldwide (Santosh et.al, 1996).[2] Feathers are made up of primarily of keratin protein and dry matter. Feathers hydrolyzed by mechanical or chemical treatment can be converted into fertilizers, feedstuffs, production of amino acids and peptides. With the help of microbial keratinolytic enzymes feathers could be converted to defined products such as rare amino acids serine, cysteine and proline. Hung et.al,(2003) stated that the proteases are the most important group of industrial enzymes and certainly form a major portion of the worldwide sales.[3] Keratin – the structural component of the integuments of birds, reptiles, amphibians etc. is a fibrous protein and a part of the skin of vertebrates and includes hair, wool, feathers, horns etc. It is an insoluble protein with very high stability and low degradation rate and belongs to the scleropeptides group. Keratins are categorized into hard keratins (hair, feather, horn, nails) and soft keratins (skin and callus) according to the sulphur content (Gupta and Ramnani, 2006).[4]

An extracellular enzyme – Keratinase is used for the bio – degradation of keratin, and can hydrolyze both native and denatured keratin. It is classified as a protease produced by keratin degrading microorganism (Letourneau et al., 1998: Bressollier et al., 1999: Friedrich et al., 1999; Singh et al., 2001).[5,6,7, 8] Keratinase is produced only in the presence of keratin substrate. Keratinolytic enzymes are widespread in nature, especially in microbial world. A vast variety of bacteria, actinomycetes and fungi are known to be keratin degraders (Wang et.al, 2003; Letourneau et.al, 1998; Santosh et.al, 1996).[9, 5, 2]

Keratinophilic fungi are generally considered as soil saprophytes. Keratinase produced by these keratinolytic fungi could be used to degrade feather waste and further the digested products could be an excellent material for producing animal feed, fertilizers or natural gas. Use of keratinolytic fungi for feather degradation is an economical, environmentally friendly alternative. As physiological and nutritional factors greatly affect feather degradation and keratinase production, the effect of various factors such as temperature and pH on feather degradation by selected fungi was reviewed in this study.

2. MATERIAL AND METHOD:

2.1 Collection of soil samples: Soil samples were collected from different animal habitats (Aviary, Serpentarium, Rabbit habitat, Guinea pig habitat, and Nocturnal house) of Kanpur Zoological Park. The samples were collected from the superficial layer of the soil collected in sterile polythene bags brought to the laboratory and stored at room temperature.

Isolation, Purification and Identification: Keratinophilic fungi were isolated by Vanbreuseghem hair bait technique.[10] Soil were placed in sterile petridishes and moistened with sterile water and baited with sterile human hairs. The petridishes were incubated at room temperature and examined after one week. After observing the growth, isolates were cultured on SDA medium at 28 ± 2^0 c for up to one week. When fungal colony is seen it is transferred to other dishes for purification. Then isolates were examined and identified on the basis of morphological characters and microscopic characters.

2.2 PREPARATION OF KERATIN SUBSTRATE (FEATHER MEAL): Chicken feather was purchased from local market and brought to the laboratory. Feather were washed many times with distilled water and dried. The dried feather were defatted by soaking in diethyl ether for 24 hours, and washed thoroughly with distilled water followed by air drying and cut into short fragments of 1-2 cm. now the processed feathers called as feather meal.

2.3 DETERMINATION OF FEATHER DEGRADATION: The utilization of feather keratin was assessed by following method of Garret (1962). Feather meal (1% feather) incubated with loop full of fungal spores in SDA broth medium for 10 days. After incubation the residual feather was determined by filtering the culture broth by taking the weight of filter paper before and after filtration. Percent reduction of feather was calculated from the difference in the initial weight and weight obtained after ten days of incubation.

2.3.1 EFFECT OF DIFFERENT TEMPERATURES ON FEATHER DEGRADATION: To determine the effect of temperature, the flask were incubated with selected isolates and feather meal at 18^0c, 28 ^{0}c, 38 ^{0}c, and 42 ^{0}c, for 10 days in static condition and filtered.

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2.3.2 EFFECT OF pH ON FEATHER DEGRADATION To determine effect of pH, the flask were adjusted to different pH i.e. 4,5,6,7,8,9 by adding the normal HCl and NaOH solution then incubated with selected isolates and feather meal for 10 days in static condition and filtered.

3. RESULTS AND DISCUSSION:

3.1 ISOLATION OF FUNGI One hundred seventy isolates yielded12 genera and 23 species of keratinophilic fungi and related dermatophytes from different indoor animal habitats of Kanpur zoological park. *Chrysosporium keratinophilum* and *Microsporum gypseum* were the most dominating species.

Fig 1: Feather meal Broth

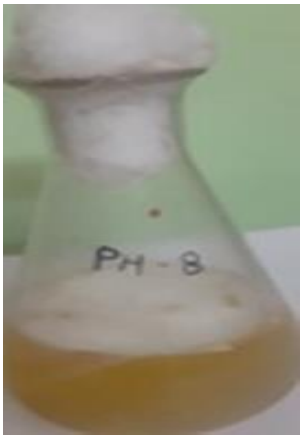

Fig 2: Growth observed in feather meal Broth

3.2 EFFECT OF DIFFERENT TEMPERATURES ON FEATHER DEGRADATION The effect of temperature on feather degradation is shown in table 1 and fig 3. It was observed that the optimum temperature was 28^0c for both the fungal isolates. The enzyme activity of *Chrysosporium keratinophilum* was lowest at the temperature below 20^0 c but gradually increased and reached maximum at 30^0c and then declined. Maximum percent reduction of feather was 56.86% at 28^0c. *Microsporum gypseum* showed best enzyme activity at 28^0c and minimum at 18^0c. Feather degradation was declined at the temperature 36^0c. Maximum percent reduction of feather was 60.78% at 28^0c. However no degradation of feather was observed at 42^0c in both the isolates.

Table 1: Feather degradation at different temperature

Fungal isolate	**Temperature (^{0}c)**	**Initial wt. of feather with filter paper (gm)**	**Final wt. of feather with filter paper (gm)**	**% reduction of feather**	**Keratinase production (U/ml**
C.keratinophilum	18	1.02	0.71 ± 0.02	30.39	47.67
	28	1.02	0.44 ± 0.005	56.86	58.90
	38	1.02	0.53 ± 0.01	48.03	53.35
	42	1.02	1.02 ±0.00	0.00	20.2
Fungal isolate	**Temperature**	**Initial wt. of**	**Final wt. of**	**% reduction of**	**Keratinase**

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	(^{0}c)	feather with filter paper (gm)	feather with filter paper (gm)	feather	production (U/ml
M. gypseum	18 28 38 42	1.02 1.02 1.02 1.02	0.58± 0.01 0.4 ± 0.011 0.48± 0.04 1.02 ± 0.00	45.09 60.78 52.94 0.00	53.24 78.62 67.0 21.8

Value is mean of three replication± Standard deviation (SD)

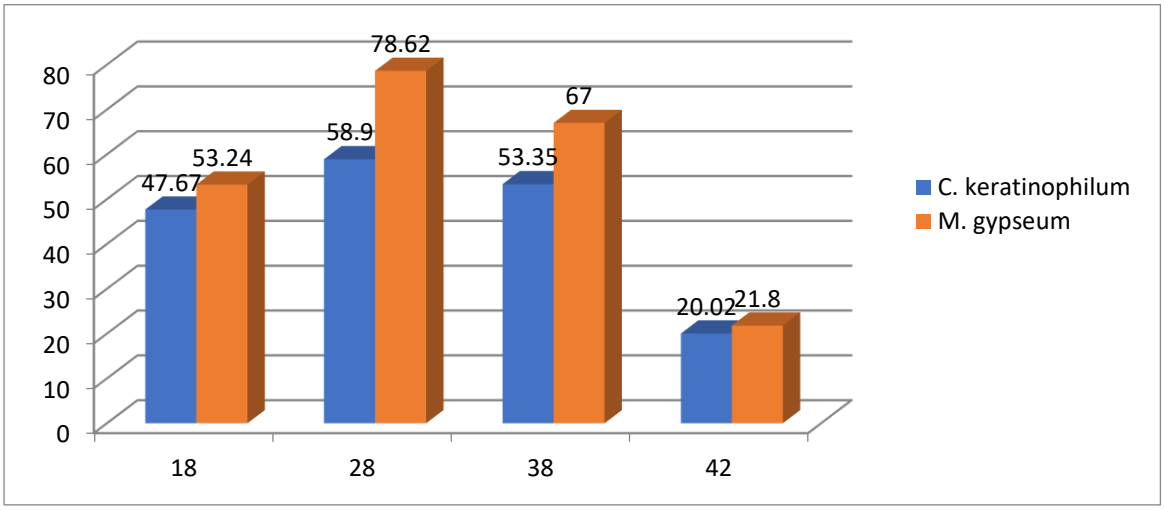

Fig 3: Effect of temperature on keratinolytic ability of isolated keratinophilic fungi

3.3 EFFECT OF pH ON FEATHER DEGRADATION The effect of pH on feather degradation is shown in table 2 and fig 4.The keratin degrading ability was found somewhat better towards alkalinity than the acidic condition. The studies of pH effect revealed that the effect of *C. keratinophilum* gradually increased from pH 6 and reached maximum in pH 8 and then decreased. A similar trend was noticed in case of *M. gypseum* .Feather weight loss was slowly increased from pH 5 and reached maximum at pH 8 then declined. The maximum percent reduction of feather was 70.58% through *C. keratinophilum* and 69.60% by *M. gypseum* at pH 8.

Table-3 Feather degradation at different pH by the isolated culture

Fungal isolate	pH	Initial wt. of feather with filter paper (gm)	Final wt. of feather with filter paper (gm)	% reduction of feather	Keratinase production (U/ml)
C.keratinophilum	4 5 6 7 8 9	1.02 1.02 1.02 1.02 1.02 1.02	0.75 0.68 0.43 0.35 0.3 0.41	26.47 33.33 57.84 65.68 70.58 58.82	32.72 46.56 60.0 64.8 68.4 60.42

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Fungal isolate	pH	Initial wt. of feather with filter paper (gm)	Final wt. of feather with filter paper (gm)	% reduction of feather	Keratinase production (U/ml)
M. gypseum	4	1.02	0.72	29.41	39.50
	5	1.02	0.63	38.23	64.12
	6	1.02	0.45	55.88	88.23
	7	1.02	0.37	63.72	110.0
	8	1.02	0.31	69.60	125.10
	9	1.02	0.42	59.80	103.82

Value is mean of three replication± Standard deviation (SD)

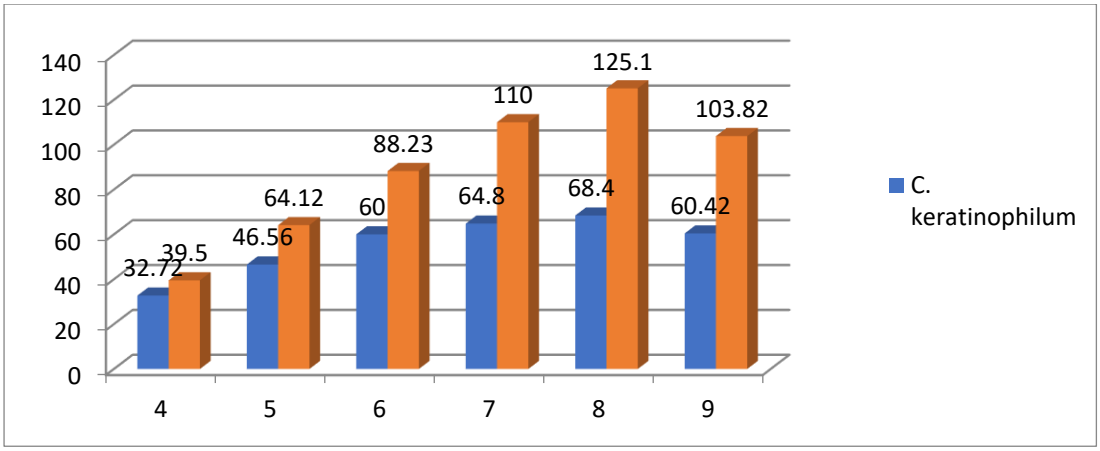

Fig4: Effect of pH on keratinolytic ability of keratinophilic fungi

4. CONCLUSION Production of feather in large amount from poultry industry is one of the major form of pollution. Traditional feather degradation reduces the overall quality of proteins and destroys essential amino acids. Biodegradation of feathers is found to be an efficient, eco-friendly, cost effective method for bioconversion of feather waste into useful products. The use of keratinolytic fungi to degrade poultry waste feather has emerged as a sustainable and alternative tool to meet this challenge.

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