African Journal of Biochemistry Research Vol. 5(1), pp. 1-6, January 2011
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2011 Academic Journals

Full Length Research Paper

In vitro biodegradation of keratin by dermatophytes and some soil keratinophiles

Mukesh Sharma*, Meenakshi Sharma and Vijay Mohan Rao

Microbiology Laboratory, Department of Botany, University of Rajasthan, Jaipur-302004, India.

Accepted 17 December, 2010

The present investigation was aimed to evaluate the *in vitro* biodegradation of keratin by clinical isolates of dermatophytes and soil fungi. Ten fungal species, out of which, six (*Chrysosporium indicum*, *Trichophyton mentagrophytes*, *Scopulariopsis* sp., *Aspergillus terreus*, *Microsporum gypseum* and *Fusarium oxysporum*) were isolated from soil and four clinical (*Trichophyton rubrum*, *Trichophyton verrucosum*, *Trichophyton tonsurans* and *Microsporum fulvum*) were obtained from human skin. The isolates were tested for their keratin degradation ability on human and animal (cow and buffalo) hair baits. The rate of keratin degradation was expressed as weight loss over three weeks of incubation. Human hair had the highest rate of keratin degradation (56.66%) by colonization of *C. indicum*. whereas *M. gypseum* and *T. verrucosum* were highly degraded (49.34%) to animal hairs. There was a significant difference ($p < 0.05$) in keratin substrate degradation rates by the examined fungi. Human hair served as an excellent source for the biodegradation of keratin by the isolated test fungi as compared to animal hair. Releasing protein showed maceration of the keratin substrates by the test fungi. The present study reveals that, the isolated test fungi play a significant impact on biodegradation of keratin substrates for betterment of environmental hazards.

Key words: Fungal species, keratin substrates, keratin degradation, released protein, environmental hazards.

INTRODUCTION

Keratin is a major component of hair, feathers and wool and is the most complex of the cytoskeletal intermediate filament proteins of epithelial cells (Latkowski and Freedberg, 1999). The durability of keratins is a direct consequence of their complex architecture. In addition to keratin, keratinaceous materials such as skin, hair, nails, hoofs and horns contain a large proportion of non-keratin proteins. A large number of fungi, including yeasts, dermatophytes and other moulds, grow on human skin, hair and nails. The term 'keratinolytic' is used for fungi exhibiting the enzymatic ability to attack and utilize keratin. Degradation of keratin by microorganisms is performed by specific proteases that is, keratinases (Onifade et al., 1998; Wang and Shih, 1999; Gradisiar et al., 2000; Sandali and Brandelli, 2000; Kim et al., 2001; Allpress et al., 2002; Longshaw et al., 2002; Yamamura et al., 2002; Gessesse et al., 2003; Singh, 2003).

Proteolytic enzymes like trypsin, pepsin and papain are largely produced in the presence of keratinous substrates in the form of hair, feather, wool, nail, horn etc. during their degradation, (Gupta and Ramnani, 2006). The complex mechanism of keratinolysis involves cooperative action of sulfitolytic and proteolytic systems. Keratinases are robust enzymes with a wide temperature and pH activity range and are largely serine or metallo proteases. A distinctive feature of keratin is its relatively high sulfur content due to the presence of sulfur containing amino acids namely cystine, cysteine and methionine. The disulphide bonds considered to be responsible for the stability of keratin and its resistance to enzymatic degradation (Kunert, 1989, 1995). Dermatophytes are often present in skin and invade the keratin tissues. Surveys of kerationophilic fungi from different habitats have indicated that, several species of dermatophytes and non-dermatophytic fungi inhabit soil (Kushwaha, 1983), air (Marchiso et al., 1994) and sewage sludge (Muhsin and Hadi, 2000). The ability of various fungal

*Corresponding author. E-mail: mukeshsharma_uniraj@yahoo.co.in.

species to degrade keratin *in vitro* from substrates such as hair (Marchiso et al., 1994; Bahuguna and Kushwaha, 1989; Deshmukh and Agrawal, 1985; Malviya et al., 1993), wool (Safranek and Goos, 1982; Al-Musalam and Radwan, 1990) and feathers (Kushwaha, 1983; El-Naghy et al., 1998; Kaul and Sumbali, 1999) have been examined.

Since soil often receives high amounts of keratin substrates due to human activities, it was of utmost interest to examine the degradation capability of the dominant fungal species in polluted soil for two common keratinaceous substrates. In the present paper, an in vitro degradation of keratin substrates by ten species of fungi isolated from soil and superficial layer of skin of patients infected with dermatomycoses has been studied and discussed.

MATERIALS AND METHODS

Ten fungal species were tested for present investigation in which six species namely *Chrysosporium indicum*, *Trichophyton mentagrophytes*, *Scopulariopsis* sp., *Aspergillus terreus*, *Microsporum gypseum* and *Fusarium oxysporum* were collected from soil and four species namely *Trichophyton rubrum*, *Trichophyton* verrucosum, *Trichophyton* tonsurans and *Microsporum fulvum* were isolated from superficial skin of human dermatomycoses patients in Jaipur (India) during 2006. The method of soil fungal isolation, identification and purification has been described previously (Vanbreuseghem, 1952; Muhsin and Hadi, 2000). The clinical fungi were isolated from dermatomycoses patients in S.M.S. Hospital, Jaipur under the able guidance of skin specialists. The fungal species were grown on Sabouraud Dextrose Agar (SDA) for two weeks. A spore suspension of each species was prepared following the method of Kunert (1989) with slight modification. In this method, a loopful spore material of each fungus was mixed separately with 1 ml of sterilized distilled water under sterilized condition. The keratin degradation by each fungal species was expressed as percentage weight loss. A total of 50 mg keratin such as human and animal hair were cut into fragments (2 cm long), washed and surface sterilized with ethanol (3%).

A buffer solution (pH 6.5) was prepared using 0.04 g of KH_2PO_4 in 100 ml distilled water in sterilized conical flasks (100 ml volume). Each flask contained 20 ml buffer solution and was inoculated with 0.2 ml of a spore suspension of each fungal species to which 50 mg of a selected substrate was added. Three replicate flasks of each fungus per substrate were prepared and incubated at 27°C in shaker incubator at 80 rpm. A control, having the keratin substrates without fungal suspension, was also run along with the test flasks. The fungal cultures were filtered after 1, 2 and 3 weeks of incubation. The keratin fragments were collected on Whatman filter paper No. 1, washed gently to remove fungal hyphae, dried at 75°C for 48 h and weighed. Changes in the pH of the culture medium with incubation time were also determined using pH meter. The protein released into the culture medium due to the keratin substrate degradation was determined (Lowry et al., 1951). During this method different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube. The final volume in each of the test tubes is 5 ml. The BSA range is 0.05 to 1 mg/ ml. From these different dilutions, pipette out 0.2 ml protein solution to different test tubes and add 2 ml of alkaline copper sulphate reagent (analytical reagent). Mix the solutions well. This solution was incubated at room temperature for 10 min. Then add 0.2 ml of reagent Folin Ciocalteau solution (reagent solutions) to each tube and incubate for 30 min. Zero the colorimeter with blank and take the optical density (measure the absorbance) at 660 nm.

Statistical analysis of data

The data collected was analyzed using one-way analysis of variance. The effects were considered significant when p value of ANOVA F-test was < 0.05.

RESULTS

The degradation rate of the human and animal hair substrates by the test fungi are presented in Figure 1. A significant difference ($p < 0.05$) in degradation rates of each keratin substrate by the fungal species was observed (Table 1). Human hair was highly colonized and degraded by *C. indicum* and *M. gypseum* at 56.66 and 49.34% of weight loss, respectively and less degraded by *Scopulariopsis* sp. at 17.34% of weight loss, over 3 weeks of incubation (Figure 1). *M. gypseum* (49.34%) and *T. verrucosum* (49.34%) showed the greatest degradation of animal hair (Table 1). These keratinous substrates exhibited low degradation by *F. oxysporum* (16.66%). Generally, there was an increase in the degradation rate of the keratin substrates, in terms of weight loss, by the fungi during incubation.

The amount of the protein released into the culture medium varied with the fungi and with different sources of keratin (Table 2). The highest amount of protein released in the cultures containing human hair was by *M. gypseum* (68 µg/ml) followed by *M. fulvum* (63 µg/ml). The lowest amount of protein released in culture medium reported by *Scopulariopsis* sp. (17 µg/ml). In cultures containing animal hair the protein released were highest in *T. verrucosum* (66 µg/ml) followed by *T. mentagrophytes* (62 µg/ml) and lowest rate of protein released was in *Scopulariopsis* sp. (13 µg/ml). Changes in pH of the culture medium due to the substrates degradation process were observed (Figures 2a and b). The pH of the control cultures did not change during the incubation periods. Keratin substrate degradation was accompanied by alkalination of the culture medium with elevation of pH from 6.5 to 8.4, when human hair was keratin substrates (Figure 2a) and 6.5 to 8.1 when animal hair was keratinous materials (Figure 2b). Present study showed erosion or perforation of the keratin substrates by the different fungal species.

DISCUSSION

The keratin substrates revealed different rates of keratin degradation with the fungi under investigation. It is generally believed that keratin degradation is due to the enzymatic action of the fungi, which is indicated by the substrate weight loss, as well as by the release of soluble products into the culture medium (Deshmukh and Agrawal, 1985). However, the distinctive feature of

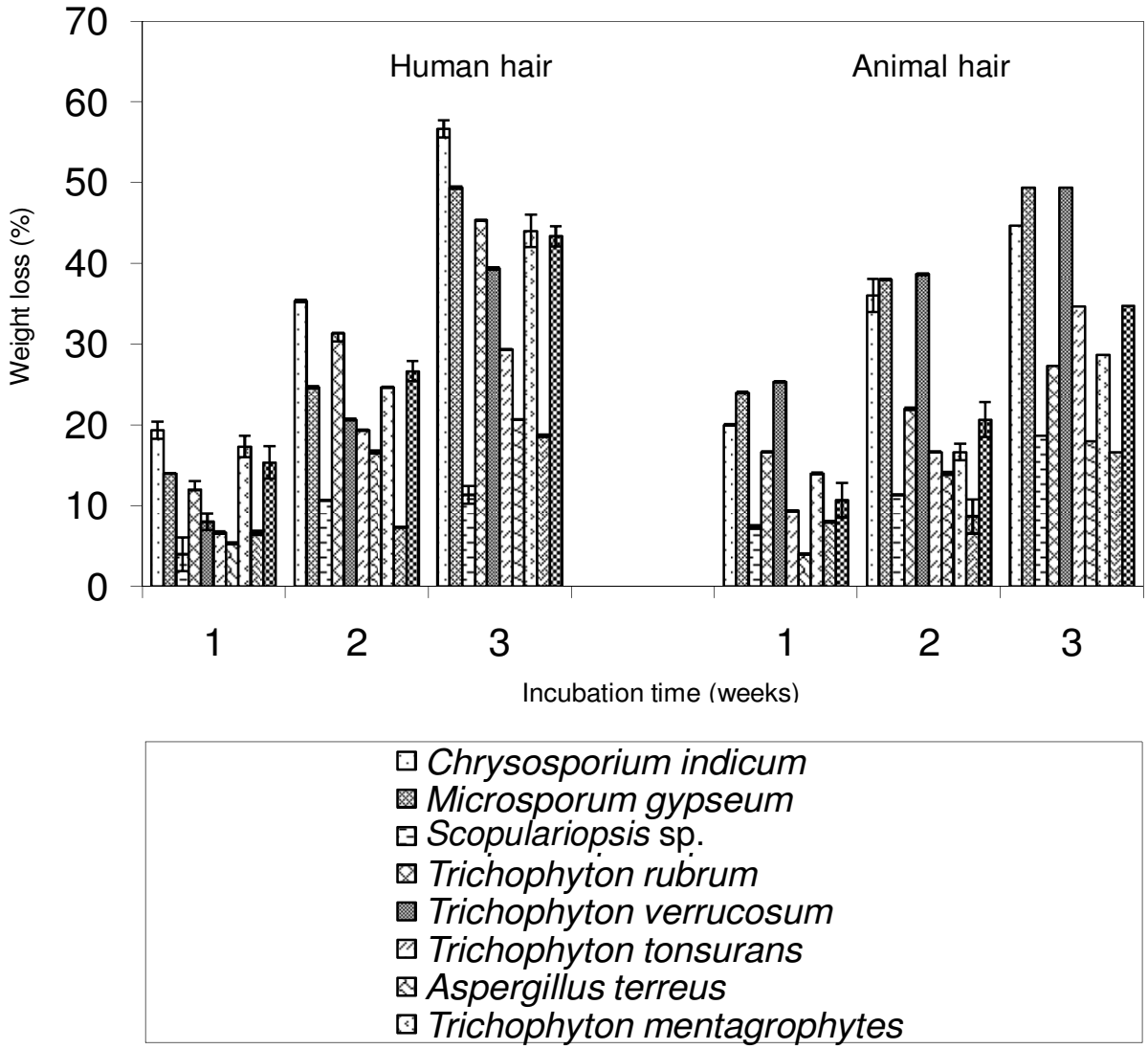

Figure 1. Degradation rate percentages of keratin substrates by fungal species during the incubation periods.

Table 1. Weight remained of keratin substrates by tested fungi in culture medium during incubation time (Initial weight = 50 mg).

Fungal species	Weight remained of keratin substrates(mg)					
	Human hair			Animal hair		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Chrysosporium indicum	40.33±0.04	32.33±0.13	21.67±0.05	40.00±0.05	32.00±0.04	27.67±0.08
Trichophyton mentagrophytes	41.33±0.15	37.67±0.41	28.00±0.09	43.00±0.13	41.67±0.05	35.67±0.12
Microsporum gypseum	43.00±0.01	37.67±0.09	25.33±0.03	38.00±0.17	31.00±0.01	25.33±0.11
Trichophyton rubrum	44.00±0.31	34.33±0.03	27.33±0.18	41.67±0.09	39.00±0.09	36.33±0.17
Trichophyton verrucosum	46.00±0.09	39.67±0.35	30.33±0.13	37.33±0.04	30.67±0.01	25.33±0.07
Trichophyton tonsurans	46.67±0.05	40.33±0.14	35.33±0.27	45.33±0.07	41.67±0.04	32.67±0.12
Microsporum fulvum	42.33±0.02	36.67±0.19	28.33±0.16	44.67±0.01	39.67±0.13	32.67±0.09
Fusarium oxysporum	46.67±0.15	46.33±0.01	40.67±0.09	46.00±0.24	45.67±0.09	41.67±0.15
Aspergillus terreus	47.33±0.09	41.67±0.16	39.67±0.01	48.00±0.05	43.00±0.16	41.00±0.16
Scopulariopsis sp.	48.00±0.03	44.67±0.11	41.33±0.09	46.33±0.03	44.33±0.13	40.67±0.05

Values are means (n=3) ± SE, the results were considered significant when $p < 0.05$.

Table 2. Protein release by tested fungi in culture medium baited with two keratin substrates.

Fungal species	Protein release (µg/ml)	
	Human hair	Animal hair
Chrysosporium indicum	57	54
Trichophyton mentagrophytes	52	59
Microsporum gypseum	68	62
Trichophyton rubrum	49	38
Trichophyton verrucosum	53	66
Trichophyton tonsurans	33	45
Aspergillus terreus	34	31
Scopulariopsis sp.	17	13
Fusarium oxysporum	29	23
Microsporum fulvum	63	51

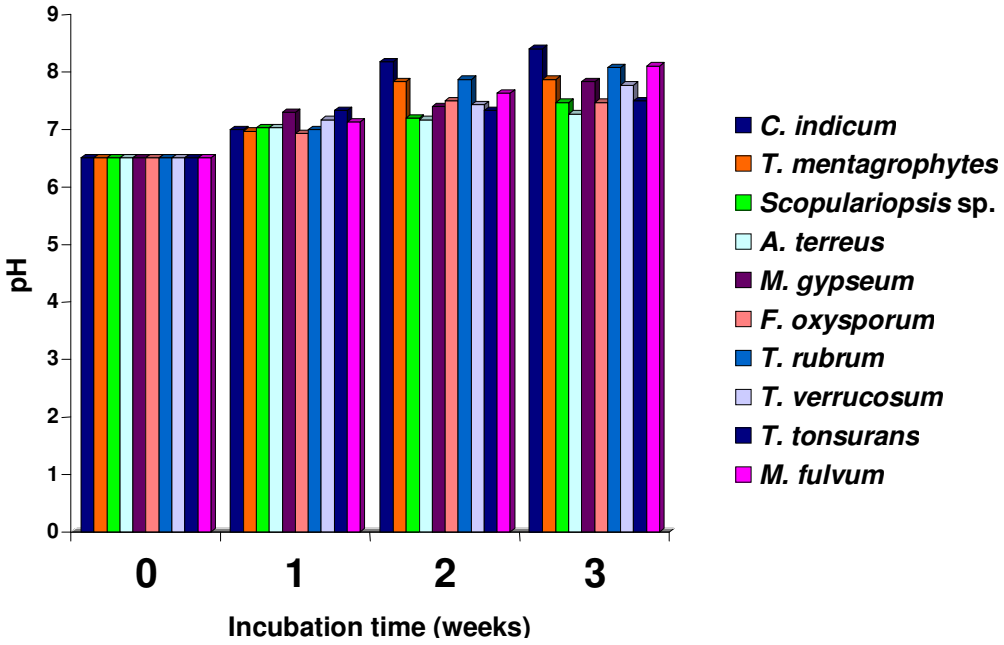

Figure 2a. pH changes of the culture medium due to keratin degradation of human hair by the fungi during the incubation periods.

keratin is its high cysteine content, that makes it more resistant to enzymatic digestion (Kaul and Sumbali, 1999). The enzyme was found to be a halophilic serine proteinase with unique substrate specificity (Namwong et al., 2006). The amount of keratinolytic enzyme in the culture fluid was dependent on the initial pH of the culture medium (Santos et al., 1996). The keratinolytic nature of the examined fungi towards different substrates may be related to their ability to produce cysteine as reported in other studies (Kunert, 1995; Kaul and Sumbal, 1999). The keratinolytic activity of dermatophytes has long attracted the attention of mycologists, biochemists and physicians. Some groups have emphasized the specific properties of fungal enzymes able to digest keratin, a substrate extremely resistant to the action of physical and chemical agents (Deshmukh and Agrawal, 1982; Kaaman and Forslin, 1985; Takatori et al., 1983). Others have isolated and characterized keratinolytic enzymes of various species of dermatophytes (Asahi et al., 1985; Sanyal et al., 1985; Takiuchi et al., 1982) and their role in virulence and the pathogenesis of mycoses has been postulated (Davies and Zaini, 1984; Eleuterio et al., 1973).

The present study has revealed that *C. indicum* has been the most active keratinolytic fungus on human hair. This species released the highest protein in medium after

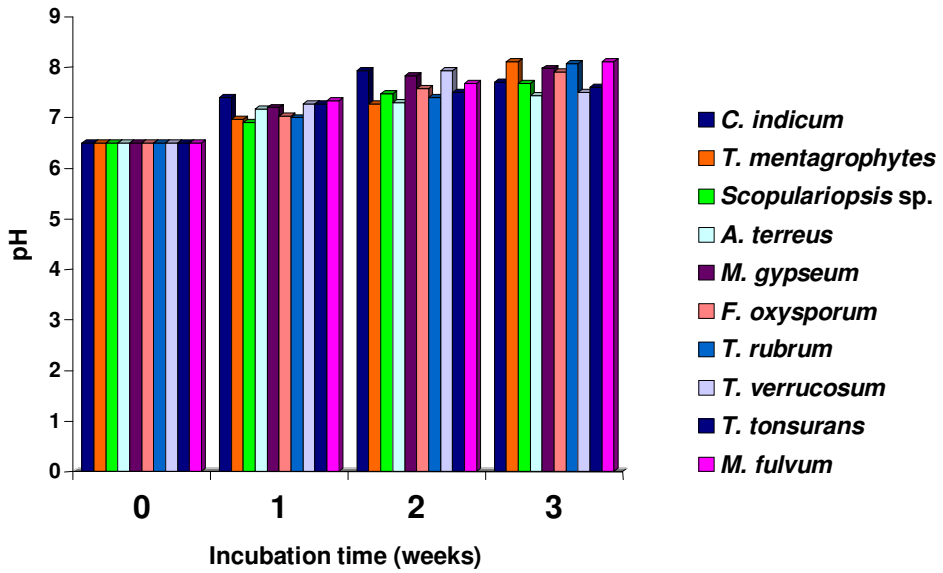

Figure 2b. pH changes of the culture medium due to keratin degradation of animal hair by the fungi during the incubation periods.

M. gypseum and *M. fulvum* respectively. Rajak et al. (1991) also studied the keratin degradation of human scalp hair by *C. indicum*, isolated from the soils of a gelatin factory in Jabalpur, India. Marchiso et al. (1991) reported the *C. indicum* was the most active keratinolytic fungi. In our study, *M. gypseum* released highest amount of protein in culture medium. This is in agreement with the study of Muhsin and Hadi (2001) who also reported that *M. gypseum* released high protein in culture medium. *Scopulariopsis* sp. and *F. oxysporum* showed the lowest ability to degrade human and animal hair and both released low levels of protein in culture. Marchiso and Fusconi (2001) studied the morphological evidence for keratinolytic activity of *Scopulariopsis* spp. isolates from nail lesions and the air. Although, *T. mentagrophytes* is known as a dermatophyte; however, it has been recorded as a human pathogen (Latkowski and Freedberg, 1999) and is frequently isolated from soil. It has also been reported that, this species is a high producer of keratinase (Muhsin et al., 1997) and proteinase (Aubaid and Muhsin, 1998). Wawrzkiewicz et al. (1991) studied *in vitro* keratinolytic activity of some dermatophytes and found that *T. verrucosum* degrade guinea pigs hair only and release enzymes mainly to the medium. In this study, *M. gypseum* and *T. verrucosum* both showed high ability to degrade animal hair in which *T. verrucosum* released high amount of protein into the culture medium followed by *M. gypseum*. The amount of keratinolytic enzyme production depended on substrates concentrations (Park and Son, 2007).

Consequently, pH of the media changed towards alkalinity. It has been stated that keratinolytic fungi often alkalinize culture media (Kaul and Sumbali, 1999). Our observation is in conformity with earlier observations (Hasija et al., 1990). It is apparent that, there are numerous nondermatophytic filamentous keratinophilic fungi belonging to diverse taxonomic groups. The ability of these fungi to invade and parasitize tissues is associated with and depends upon use and breakdown of keratin. A great variety of non-dermatophytic filamentous fungi can utilize keratin for their growth and are strongly keratinolytic with no concrete evidence of their pathogenic role; their mere isolation in culture from lesions on skin or other sites should not ascribe them any etiological significance. Thus, it can be concluded that fungi isolated by us, which are keratinophilic in nature play an important role not only in pathogenicity but also in biodegradation of keratin substrates.

ACKNOWLEDGEMENTS

The authors are grateful to the Head, Department of Botany, University of Rajasthan, Jaipur, India for providing research facilities and to Dr. V. N. Saxena, S.M.S. Medical College and Hospital, Jaipur, India for providing the help in isolation of dermatophytic fungi from dermatomycoses patients.

REFERENCES

Allpress JD, Mountain G, Gowland PC (2002). Production, purification and characterization of an extracellular keratinase from Lysobacter NCIMB 9497. Lett. Appl. Microbiol., 34: 337-342.

Al-Musalam AA, Radwan SS (1990). Wool-colonizing microorganisms capable of utilizing wool-lipids and fatty acids as sole sources of carbon and energy. J. Appl. Bacteriol., 69: 806-813.

Asahi M, Lindquist R, Fukuyama K, Apodaca G, Epstein WL, Mckerrow JH (1985). Purification and characterization of major extracellular

proteinases from Trichophyton rubrum. Biochem. J., 232: 139-144.
Aubaid AH, Muhsin TM (1998). Partial purification and kinetic studies of exocellular proteinase from Trichophyton mentagrophytes var. erinacei. Mycoses, 41: 163-168.
Bahuguna S, Kushwaha RKS (1989). Hair perforation by keratinophilic fungi. Mycoses, 32: 340-343.
Davies RR, Zaini F (1984). Enzymatic activities of Trichophyton rubrum and the chemotaxis of polymorphonuclear leucocytes. Sabouraudia, 22: 235-141.
Deshmukh SK, Agrawal SC (1982). In vitro degradation of human hair by some keratinophilic fungi. Mykosen, 25: 454-458.
Deshmukh SK, Agrawal SC (1985). Degradation of human hair by some dermatophytes and other keratinolphilic fungi. Myksosen, 28: 463-466.
Eleuterio MK, Grappel SF, Caustic CA, Blank F (1973). Role of keratinases in dermatophytosis. III. Demonstration of delayed hypersensitivity to keratinases by the capillary tube migration test. Dermatologica, I47: 255-260.
El-Naghy MA, El-Katany E, Fadl-Allah EM, Nazeer WW (1998). Degradation of chicken feathers by Chrysosporium georgiae. Mycopathologia, 143: 77-84.
Gessesse A, Hatti-Kaul R, Gashe BA, Mattiasson B (2003). Novel alkaline proteases from alkaliphilic bacteria grown on chicken feather. Enzyme Microb. Technol., 32: 519-524.
Gradisiar H, Kern S, Friedrich J (2000). Keratinase of Doratomyces microsporus. Appl. Microbiol. Biotechnol., 53: 196-200.
Gupta R, Ramnani P (2006). Microbial keratinases and their prospective applications: An overview. Appl. Microbiol. Biotechnol., 70(1): 21-33.
Hasija SK, Malviya H, Rajak RC (1990). Keratinolytic ability of some fungi isolated from gelatin factory campus, Jabalpur (MP). Proc. Nat. Acad. Sci. India, 3: 305-309.
Kaaman T, Forslind B (1985). Ultrastructural studies on experimental hair infections *in vitro* caused by *Trichophyton mentagrophytes* and *T. rubrum.* Act. Dermatologica. Venereologica (Stock), 65: 536-569.
Kaul S, Sumbali G (1999). Production of extracellular keratinase by keratinophilic fungal species inhabiting feathers of living poultry birds (Gallus domesticus): A comparison. Mycopathologia, 146: 19-24.
Kim JM, Lim WJ, Suh HJ (2001). Feather-degrading *Bacillus* species from poultry waste. Process. Biochem., 37: 287-291.
Kunert J (1989). Biochemical mechanism of keratin degradation by the actinomycete Streptomyces fradiae and the fungus Microsprum gypseum: A comparison. J. Basic Microbiol., 9: 597-604.
Kunert J (1995). Biochemical mechanism of keratin degradation by keratinolytic fungi. In: Govil, C.M., Bilgrami, K.S., Singh, D. (eds). Platinum jubilee volume of Indian Botanical Society. Agrawal Printers Meerut, India, pp. 89-98.
Kushwaha RKS (1983). The *in vitro* degradation of peacock feathers by some fungi. Mykosen, 26: 324-326.
Latkowski JM, Freedberg IM (1999). Epidermal cell kinetics, epidermal differentiation, and keratinization. In: Freedberg, I.M., Eisen, A.Z., Wolff, K. (eds). Fitzpatrick's Dermatology in General Medicine. McGraw-Hill, New York, pp. 133-144.
Longshaw CM, Wright JD, Farrell AM, Holland KT (2002). Kytococcus sedentarius, the organism associated with pitted keratolysis, produces two keratin-degrading enzymes. J. Appl. Microbiol., 93: 810-816.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
Malviya HK, Rajak RC, Hasija SK. (1993). *In vitro* degradation of hair keratin by Graphium penicilloides; Evidence for sulphytolysis and peptidolysis. Crypto. Bot., 3: 197-201.
Marchiso VF, Fusconi A (2001). Morphological evidence for keratinolytic activity of *Scopulariopsis* spp. isolates from nail lesions and the air. Med. Mycol., 39 (3): 287-294.
Marchiso VF, Curetti D, Cassinelli C, Bordese C (1991). Keratinolytic and keratinophilic fungi in the soils of Papua New Guinea. Mycopathologia, 115(2): 113-119.
Marchiso VF, Fusconi A, Rigo S (1994). Keratinolysis and its morphological expression in hair digestion by airborne fungi. Mycopathologia, 127: 103-115.
Muhsin TM, Aubaid AH, Al-Duboon AH (1997). Extracellular enzyme activities of deramatophytes and yeast isolates on solid media. Mycoses, 40: 465-469.
Muhsin TM, Hadi RB (2000). Dermatophytes and other keratinolytic fungi from sewage sludge at Basrah, Iraq. Mycoses (in press).
Muhsin TM, Hadi RB (2001). Degradation of keratin substrates by fungi isolated from sewage sludge. Mycopathologia, 154: 185-189.
Namwong S, Hiraga K, Takada K, Tsunemi M, Tanasupawat S, Oda K (2006). A halophilic serine proteinase from Halobacillus sp. SR5-3 isolated from fish sauce: purification and characterization. Biosci. Biotechnol. Biochem., 70(6): 395-401.
Onifade AA, Al-Sane NA, Al-Mussallam AA, Al-Zarbam S (1998). Potential for biotechnological application of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. Biores. Technol., 6: 1-11.
Park GT, Son HJ (2007). Keratinolytic activity of Bacillus megaterium F7-1, a feather-degrading mesophilic bacterium. Microbiol. Res., 23, [Epub ahead of print].
Rajak RC, Parwekar S, Malviya H, Hasija SK (1991). Keratin degradation by fungi isolated from the grounds of a gelatin factory in Jabalpur, India. Mycopathologia, 114(2): 83-87.
Safranek WW, Goos RD (1982). Degradation of wool by saprophytic fungi. Can. J. Microbiol., 28: 137-140.
Sandali S, Brandelli A (2000). Feather keratin hydrolysis by a *Vibrio sp.* strain kr2. J. Appl. Microbiol., 89: 735-743.
Santos RMDB, Firmino AA, De Sa CM, Felix CR (1996). Keratinolytic activity of Aspergillus fumigatus Fresenius. Curr. Microbiol., 33(6): 364-370.
Sanyal AK, Das SK, Banerjee AB (1985). Purification and partial characterization of an excellular proteinase from Trichophyton rubrum. Sabouraudia, 23: 165-178.
Singh CJ (2003). Optimization of an extracellular protease of Chrysosporium keratinophilum and its potential in bioremediation of keratinic wastes. Mycopathologia, 156: 151-156.
Takatori K, Udagawa S, Kurata H, Hasagawa A (1983). Microscopic observation of human hairs infected with Microsporum ferrugineum. Mycopathologia, 81: 129-133.
Takiuchi I, Higuchi D, Sei Y, Koga M (1982). Isolation of an extracellular proteinase (keratinase) from Microsporum canis. Sabouraudia, 20: 281-288.
Vanbreuseghem R (1952). Biological technique for the isolation of dermatophytes from the soil. Ann. Soc. Belge. Med. Trop., 32: 173-178.
Wang JJ, Shih JCH (1999). Fermentation production of keratinase from Bacillus licheniformis PWD-1 and a recombinant *B. subtilis* FDB-29. J. Ind. Microbiol. Biotechnol., 22: 608-616.
Wawrzkiewicz K, Wolski T, Lobarzewski J (1991). Screening the keratinolytic activity of dermatophytes *in vitro*. Mycopathologia, 114(1): 1-8.
Yamamura S, Morita Y, Hasan Q, Yokoyama K, Tamiya E (2002). Keratin degradation: A cooperative action of two enzymes from *Stenotrophomonas* sp. Biochem. Biophys. Res. Commun., 294: 1138-1143.