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Keratinases and microbial degradation of Keratin

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ABSTRACT

The present review deals with fungal keratinases including that of dermatophytes. Bacterial keratinases were also included. Temperature and substrate relationship keratinase production has also been discussed. Keratin degradation and industrial involvement of keratinase producing fungi is also reviewed.

Key words: Keratinase, keratin, degradation, fungi.

INTRODUCTION

Keratin is an insoluble macromolecule requiring the secretion of extra cellular enzymes for biodegradation to occur. Keratin comprises long polypeptide chains, which are resistant to the activity of non-substrate-specific proteases. Adjacent chains are linked by disulphide bonds thought responsible for the stability and resistance to degradation of keratin (Safranek and Goos, 1982). The degradation of keratinous material is important medically and agriculturally (Shih, 1993; Matsumoto, 1996). Secretion of keratinolytic enzymes is associated with dermatophytic fungi, for which keratin is the major substrate (Matsumoto, 1996). However, the production of such enzymes is not exclusive to dermatophytes, since geophilic species have demonstrated keratinase production (Kushwaha and Nigam, 1996). World-wide poultry processing plants produce millions of tons of feathers as a waste product annually (Santos et al. 1996), which consists of approximately 90% keratin; the keratin is largely responsible for their high degree of recalcitrance.

The keratinous wastes is increasingly accumulating in the environment mainly in the form of feathers, hair, horns, hooves and nails generated from various industries. The sewage and bottom sediments of rivers and canals contains contains an enormous amount of hidden keratinous waste as a result of daily shaving habits in metros. Today, it is also becoming a part of solid waste management and it is difficult to degrade. Recycling of such wastes is increasing attention. Keratin forms a major component of the epidermis and its appendages viz. hair, feathers, nails, horns, hooves, scales and wool. On the basis of secondary structural confirmation, keratins have been classified into α and β (Voet and Voet, 1995; Akhtar and Edwards, 1997). The keratin fibrils in both the configurations are twisted in a parallel manner to form micro and macro fibrils that warrant stability to the fiber (Kreplak et al. 2004; Zerdani et al. 2004). Keratins are also grouped into hard and soft keratins according to the sulfur content. Hard keratins found in appendages like feathers, hair, hooves and nails have high disulfide bond content and are tough and inextensible. Whereas, soft keratins like skin have low content of disulfide bonds and are more pliable (Voet and Voet, 1995; Schrooyen et al. 2001). Keratin wastes can be efficiently degraded by bacteria, actinomycetes and fungi due to keratinases (Onifade et al. 1998). Keratinases known to date cannot completely solubilize native keratin (Ignatova et al. 1999; Ramnani et al. 2005). Nonetheless, keratinases in nature have been continuously contributing to valorization of voluminous keratin containing wastes in the form of hair, feathers, dead birds and

animals (Onifade et al. 1998; Farag and Hassan, 2004). Keratinases from dermatophytic fungi have long been well known due to their notorious pathogenic nature (Sohnle and Wagner, 2000), these enzymes have only recently gained biotechnological importance. Their growing importance is mainly contributed to the isolation of keratinases from non-pathogenic microorganisms and their ability to degrade keratin into economically useful keratin product (Onifade et al. 1998; Lin et al. 1999; Riffel et al. 2003), nitrogenous fertilizers, biodegradable films, glues and foils (Friedrich and Antranikian, 1996; Schrooyen et al. 2001; De Toniet et al. 2002).

However, they also represent a potentially valuable source of protein as animal feedstock if keratinolysis can be achieved (Shih, 1993). Keratinolytic enzymes have been studied from a variety of fungi and, to a lesser extent, bacteria. However, current research is centered on the potential use of keratinases of bacterial origin for the industrial treatment of keratin-containing compounds, e.g. serine proteases produced by *Bacillus licheniformis* (Lin et al. 1997, Evans et al. 2000). Such interest results from the broad substrate range of these bacterial enzymes, their rates of activity towards keratin-containing compounds and their thermo stability.

Filamentous fungi synthesize a variety of hydrolytic enzymes. Several species are used for the production of industrially important enzymes such as different proteases, carbohydrases and lipases. They are the key enzymes in fungal invasion of skin and skin formations and have been mostly studied for dermatophytes such as Trichophyton [Yu et al. 1968] and *Microsporum* (Takiuchi et al 1982, 1984) as well as the yeast *Candida* which also causes skin infections. Keratinolytic enzymes are also involved in microbial bioconversion of keratinous waste. For this purpose, enzymes of *Streptomyces* (Bockle et a. 1995 and *Bacillus* spp. (Lin et al 1992, 1995) have been investigated. Noval and Nickerson (1959) tested 15 bacteria, 21 actinomycetes and 18 fungi for keratinolytic activity and found that *Streptomyces* was most effective in the degradation of sheep wool. Among 34 fungi tested for keratin hydrolysis, the most active in peacock feathers were *Verticillium tenuipes, Trichophyton equinum* and *T. mentagrophytes.* In another study among 21 fungi tested for degrading chicken feathers, *Trichophyton simii* was most effective. In test on different keratins, 16 dermatophytes were tested. Guinea pig hair was degraded by *T. mentagrophytes, T. verrucosum* and *Keratinomyces ajelloi*, while only *T. gallinae* was able to degrade chicken feathers (Warwrzkiewicz et al. 1991)

FUNGAL KERATINASE

Acremonium, Alternaria radicina, Aspergillus flavus, Cladosporium cladosporoides, Curvularia inequalis, Fusarium culmorum, Geotrichum sp. Gliomastrix murorum, Monodactys castannea, Myrothecium verrucaria, Pacecilomyces variotii, Penicillim raistrckii, Stachybotrys atra, Trichurus spiralis and *Ulocladium botrytis* were studied by Fredrich et al. (1999) for the keratinase production.

Scott and Untereiner (2004) screened following fungi for their ability to produce keratinase employing keratin azure method. *Arthroderma curreyi, A. gypseum, A. incurvatus, A. otae, A. quadrifidum, A. silverae, Chrysosporium vallenarense, Ctenomyces serratus, Epidermophyton floccosum, Milochevitch Microsporum canis, M. cookei, M. persicolor, Trichophyton krajdenii, T. mentagrophytes, T. mentagrophytes, T. mentagrophytes, T. mentagrophytes, T. raubitschekii, T. rubrum, T. simii, Arachniotus ruber, Arachnomyces minimus, Gymnascella aurantiaca, Gymnoascoideus petalosporus, Gymnoascus reessii, Amauroascus aureus, A. mutatus, A. niger, A. purpureus, Aphanoascus fulvescens, A. mephitalis, A. terreum, Apinisia graminicola, Ascocalvatia alveolata, Auxarthron californiense, A. zuffianum, Chrysosporium keratinophilum, C. tropicum, Nannizziopsis vriesii, Neogymnomyces demonbreunii, Onygena equina, Polytolypa hystricis, Renispora flavissima, Shanorella spirotricha, Spiromastix grisea, S. tentaculatum, S. warcupii, S. warcupii, Uncinocarpus reesii, Aspergillus alliaceus, A. niger, Byssochlamys nivea.* The most keratinolytic group among fungi belongs to fungi imperfectii including the following genera: *Chrysosporium, Aspergillus, Alternaria, Trichurus, Curvularia, Cladosporium, Fusarium, Geomyces, Gleomastis, Monodictys, Myrothecium, Paecilomyces, Stachybotrys, Urocladium, Scopulariopsis, Sepedonium, Penicillium, Doratomyces.*

Four fungal species including two dermatophytes and two saprophytes were isolated from sewage sludge samples, they were tested for their degradative ability towards three types of keratin substrates (human hair, chicken feathers and wool). The rate of keratin degradation was expressed as weight loss over three weeks of incubation using a liquid culture medium. Human hair had the highest degradation rate by colonization of *Chrysosporium pannicola* and *Microsporum gypseum* at a rate of 62% and 4% respectively. Chicken feathers were highly degraded by *Aspergillus flavus* (32%) while wool degradation was highest by *C. pannicola* (45.5%) and *Trichophyton mentagrophytes* var. *erinacei* (38%). There was a significant difference ($p < 0.00l$) in keratin substrate degradation

rates by the examined fungi. Keratinase activity was highest for *C. pannicola* and *M. gypseum* in the culture medium baited with human hair. *Aspergillus flavus* revealed the highest activity of this enzyme in cultures amended with chicken feathers while *T. mentagrophytes* var. *erinacei* showed highest keratinase activity in cultures with wool substrate. The amount of protein released into the culture medium varied among the tested fungi. The medium's alkalinity increased over incubation time from 6.5 to 7.8. Microscopic examination showed maceration of the keratin substrates by the fungi.

Fungal involvement in keratin degradation and keratinase production was shown by many workers. (Evans and Hose, 1975; Safranek and Goos, 1982; Nigam and Kushwaha, 1992; Kushwaha and Nigam, 1995; Kushwaha, 1995, 2000). Extra cellular and cell bound keratinases of *T. mentagrophytes* were studied by Yu et al. 1967, 1971. Calvo et al. 1991 tested 300 strains of Chrysosporium for keratinase and other proteases. Keratin degradation ability of *Aspergillus quercinus, Chrysosporium tropicum, Microsporum fulvum* and *Trichophyton mentagrophytes* was determined by Kushwaha and Agrawal (1981). Feather and leather degadation by 5 *Chrysosporium*, 4 *Aspergillus*, 2 *Penicillium* and 2 each of *Acremoniun* and *Fusarium* was monitored by Nigam et al. (1994). In a study 71 soil fungal isolates of 20 *Acremonium* and 51 *Chrysosporium* isolates were screened for protein release and keratinase production (Bhadauria and Kushwaha, 2002) and all these showed high amount of protein and keratinase (Table 1). Twenty different geophilic fungi of different genera when grown on pig hair (Kushwaha and Nigam,1996), pecock feathers (Kushwaha, 1983) and chicken feathers + human hair (Kushwaha, 2007) produced keratinases (Table 2) indicating that different substrates induces different amount of keratinase. It is worthy to note that when a mixture of equal amount of chicken feathers and human hairs are used as substrate the amont of keratinase increased. Singh and Kushwaha (2006) illustrated the hair penetration by keratinophilic fungi. *Chrysosporium georgii* has also been able to produce intracellular and extra cellular keratinses.

KERATINASE AND DERMATOPHYTES

The dermatophytes are a group of fungi that can invade the keratinized tissues of human and animals. The ability of these fungi to produce various enzymes has been implicated in the pathogenicity of the host skin (Matsumata, 1996). Keratinase is the major enzyme involved in the pathogenesis process (Howard, 1983) and noteworthy information is available on the keratinase production by different species of dermatophytes (Takuchi, et al 1984, Wawarzkiez, 1991, Quin, et al 1992). Among the dermatophytes, different species of *Trichophyton* were reported as exocellular enzyme producers (Samadani et al, 1995; Ibrahim- Granet; 1996). It is of interest to study the enzyme patterns of dermatophytes particularly the keratinase as this plays an important role in the break down of keratin substrate. Since keratin is the main component of the skin, hair and nails, the break down of this substrate by dermatophytes requires a secretion of a specific enzyme. The present study demonstrated that keratinase produced by *Trichophyton mentagrophytes* var. *erinacei*can actively digest guinea pig hair and fibrous protein as substrates. Thus this dermatophyte is highly capable to invade the keratinized tissues. A comparison of keratinase released by *Trichophyton mentagrophytes* with other related species showed a great similarity in the enzyme characterization with that of *T. schoenlenii* as both dermatophytes having a MW of 38 000 dalton. However, the activity of keratinase produced by *T. schoenlenii* was found to be increased against guinea pig hair in culture medium amended with Fe. Brasch et al. (1991) showed that when keratin is supplied into the medium seven different enzymes are released by *T. rubrum* which indicated that the extra cellular enzymes activity dependent on the nutrient supplied into the growth medium. The optimal keratinase activity was detected at pH 5.5 and its stability was at 55°C indicating that the high activity of the enzyme occurred at an acidic medium as previously stated (Muslin, et al. 1997). It is recommended that the enzyme assay should be established under certain pH and temperature. Nevertheless, the high activity of keratinase in guinea pig hair and fibrous protein can be related to its substrate specificity or due to the removal of some accessory proteins that are capable of splitting the disulphide bonds present in keratinized protein during the purification process. On the other hand, the enzyme activity was inhibited by PMSF and less inhibited by NEM may explain that the purified keratinase 125 belongs to the acidic protein group as suggested before (Simpany and Baxter, 1996). A recent study on proteinase produced by *Trichophyton entagrophytes* var. *erinacei* showed that this enzyme belongs to a serine group (Aubaid and Mushin, 1998). Purified keratinase showed no inhibition in the presence of the serine protease inhibitor PMSF. However, inhibition was demonstrated in the presence of EDTA, indicating that the keratinase is a metalloprotease. The majority of the keratinases reported in the literature have been shown to be serine proteases. A metalloprotease was reported to be produced extracellularly by *Streptomyces pactum* (Bo¨ckle et al. 1995). However, a serine protease produced in conjunction with the metalloprotease was largely responsible for keratinolytic activity in this strain.

Among potential virulence factors of dermatophytes, secreted proteases, and especially keratinolytic ones, have been investigated the most. They could provide the fungus with nutrients, by degrading keratin into easily assimilable metabolites (Apodaca and McKerrow, 1989a), and allow the invasion of keratinized structures (Apodaca and McKerrow, 1989b). Furthermore, keratinolytic proteases could be involved in the control of host defense mechanisms (Grappel and Blank, 1972; Collins et al. 1973). Keratinolytic proteases have been isolated from different species of dermatophytes including *Trichophyton rubrum* (Meevootisom and Niederpruem, 1979; Asahi et al. 1985; Lambkin et al. 1996), *Trichophyton mentagrophytes* (Yu et al. 1968,1971; Tsuboi et al. 1989), and *M. canis* (Takiuchi et al. 1982,1984; Lee et al. 1987; Mignon et al. 1998; Brouta et al. 2001). Despite the presumed role of these keratinases in pathogenesis, very few studies have dealt with their *in vivo* expression. A well-characterized 31.5 kDa keratinolytic subtilisin-like serine protease was previously shown to be secreted by *M. canis* as the major component, *in vitro*, in a minimal medium enriched with cat keratin (Mignon et al. 1998). Moreover, the *in vivo* expression of this keratinase was demonstrated in hair of both *M. canis* naturally infected cats (Mignon *et al*, 1998b) and experimentally infected guinea pigs (Mignon et al 1999), suggesting its role in the pathogenesis of this dermatophytic infection. The role of this protease, thought to be an essential *M. canis* virulence factor, should be further investigated, however no dermatophyte keratinolytic protease has been characterized so far at the molecular level. Such a characterization would therefore be an important step towards the understanding of dermatophytic infection pathogenesis.

The amino acid sequence of the *M. canis*-secreted SUBs also showed high percentages of identity with secreted proteases from the filamentous fungi *T. rubrum* (Woodfolk et al 1998), *A. fumigatus* (Jaton-Ogay et al 1992), *A. oryzae* (Tatsumi et al. (1988), *A. flavus* (Ramesh *et al* (1994), and *A. nidulans* (Katz et al. 1994). Other sequence homologies between *M. canis* and *Aspergillus* spp. proteases have already been reported. Indeed, the N-terminal extremity sequence of the 43.5 kDa *M. canis* keratinolytic metalloprotease (Brouta et al. 2001) disclosed marked similarities with those of metalloproteases from *A. fumigatus* (Monod et al. 1993) and *A. oryzae* (Doumas et al. 1999). Moreover, the recent molecular characterization of the 43.5 kDa *M. canis* keratinase gene showed that it was homologous to genes encoding the latter metalloproteases (Brouta et al. unpublished). These results strengthen the hypothesis (Brouta et al. 2001) according to which *M. canis* and *Aspergillus* spp., both members of the *Onygenales* family, would share fundamental similarities in their proteolytic system, even though they produce proteinases with different specificities related to the substrates they hydrolyze and to the tissues they can invade.

BACTERIAL KERATINASES

Keratinolytic enzymes are widespread in nature and are elaborated by a numerous microorganisms isolated from different habitats and sources. A vast variety of bacteria, actinomycetes and fungi are able to degrade keratin. Among bacteria, degradation is mostly confined to gram-positives, including *Bacillus, Lysobacter, Nesternokia, Kocurica* and *Microbacterium.* However, a few strains of gram-negative bacteria, viz. *Vibrio, Xanthomonas, Stenotrophomonas* and *Chryseobacterium* (Sangali and Brandelli, 2000; De Toni et al. 2002; Yamamura et al. 2002 ; Lucas et al. 2003), have also been recently reported. In addition, a few thermophiles and extremophiles belonging to the genera *Fervidobacterium, Thermoanaerobacter, Bacillus* and *Nesternokia* have also been described (Friedrich and Antranikian 1996; Rissen and Antranikian, 2001). Actinomycetes from the Streptomyces group, viz. *S. fradiae* (Novel and Nickerson, 1959), *Streptomyces* sp. (Mukhopadhyay and Chandra, 1990), *S. pactum* (Bockle et al. 1995), *S. albidoflavus* (Letourneau et al. 1998), *S. thermoviolaceus* (Chitte et al. 1999) and *S. graminofaciens* (Szabo et al. 2000), and the *Thermoactinomyces* group, viz. *T. candidus* (Ignatova et al. 1999) and another *Thermoactinomyces* sp. (Gousterova et al. 2005), is also reported as keratin degraders. However, only a few have reached commercial exploitation. Keratinases from *Bacillus* sp. particularly *B. licheniformis* and *B. subtilis* have been extensively studied due to their effectiveness in terms of feather degradation (Manczinger et al. 2003; Thys et al. 2004). B. licheniformis, a source of the Versazyme, the first commercial keratinase developed by Shih and coworkers at North Carolina. Industrial trials are ongoing and the product is expected to reach the market shortly (Gregg, 2002).

Keratin serves as the inducer; however, soy meal is also known to induce enzyme production (Gradisar et al. 2000). Most of the reports available on keratinases group them as inducible enzymes; however, few constitutive keratinases have also been reported (Manczinger et al. 2003). Microbial keratinases are predominantly extra cellular when grown on keratinous substrates; however, a few cell-bound (Friedrich and Antranikian, 1996; Onifade et al. 1998; Rissen and Antranikian, 2001; Nam et al. 2002) and intra cellular keratinases have also been reported (El-Naghy et al. 1998; Onifade et al. 1998).

Table1. Protein released from bird feathers during growth of keratinophilic fungi

S. no.	Fungi tested	Accession number	Total* protein [µg/ml]	Keratinase Ku/ml
1.	*Acremonium* sp.1	GPCK 506	366.99	115.8
2.	*Acremonium* sp.2	GPCK 537	494.33	117.6
3.	*Acremonium* sp.3	GPCK 538	441.33	116.5
4.	*Acremonium* sp.4	GPCK 539	417.66	115.2
5.	*Acremonium* sp.5	GPCK 540	312.33	116.6
6.	*Acremonium* sp.6	GPCK 541	351.00	114.5
7.	*Acremonium* sp.7	GPCK 542	313.99	112.3
8.	*Acremonium* sp.8	GPCK 543	269.66	110.5
9.	*Acremonium* sp.9	GPCK 544	215.33	110.2
10.	*Acremonium* sp.10	GPCK 545	213.66	110.1
11.	*Acremonium* sp.11	GPCK 546	196.99	76.9
12.	*Acremonium* sp.12	GPCK 547	189.66	69.2
13.	*Acremonium* sp.13	GPCK 548	87.32	16.5
14.	*Acremonium* sp.14	GPCK 549	147.99	88.8
15.	*Acremonium* sp.15	GPCK 550	107.66	18.2
16.	*Acremonium* sp.16	GPCK 551	106.00	17.5
17.	*Acremonium* sp.17	GPCK 640	158.33	114.6
18.	*Acremonium implicatum*	FMR 6212	187.33	115.3
19.	*Acremonium hennebertii*	FMR 6213	195.33	118.6
20.	*Acremonium hennebertii*	FMR 6214	185.66	114.2
26.	*Chrysosporium europae*	FMR 300	457.33	116.6
27.	*Chrysosporium cuniculi*	GPCK 673	231.66	114.2
28.	*Chrysosporium indicum*	GPCK 502	132.00	17.8
29.	*Chrysosporium indicum*	GPCK 639	110.99	16.6
30.	*Chrysosporium indicum*	GPCK 648	154.33	18.5
31.	*Chrysosporium indicum*	GPCK 649	300.33	115.3
32.	*Chrysosporium indicum*	GPCK 650	195.99	110.9
33.	*Chrysosporium indicum*	GPCK 651	228.33	111.6
34.	*Chrysosporium indicum*	GPCK 652	294.99	115.8
35.	*Chrysosporium indicum*	GPCK 653	123.99	110.2
36.	*Chrysosporium indicum*	GPCK 654	156.00	111.5
37.	*Chrysosporium indicum*	GPCK 655	241.32	112.4
38.	*Chrysosporium indicum*	GPCK 656	173.66	110.0
39.	*Chrysosporium indicum*	GPCK 657	106.66	17.2
40.	*Chrysosporium indicum*	ITCC 4730	184.66	112.0
41.	*Chrysosporium keratinophilum*	GPCK 501	185.99	75.0
42.	*Chrysosporium keratinophilum*	GPCK 614	384.33	115.9
43.	*Chrysosporium keratinophilum*	GPCK 658	416.99	118.8
44.	*Chrysosporium keratinophilum*	GPCK 659	151.66	115.1
45.	*Chrysosporium keratinophilum*	GPCK 660	137.66	110.0
46.	*Chrysosporium keratinophilum*	GPCK 661	397.33	112.0
47.	*Chrysosporium keratinophilum*	GPCK 662	345.66	115.4
48.	*Chrysosporium keratinophilum*	GPCK 663	172.99	48.8
49.	*Chrysosporium keratinophilum*	GPCK 773	107.99	37.9
50.	*Chrysosporium keratinophilum*	ITCC 4729	179.33	110.3
51.	*Chrysosporium keratinophilum*	P 318	141.33	114.5
52.	*Chrysosporium pannicola*	GPCK 612	140.66	114.1
53.	*Chrysosporium pannicola*	GPCK 670	354.32	116.6
54.	*Chrysosporium pannicola*	GPCK 671	114.33	111.1
55.	*Chrysosporium pannicola*	GPCK 672	179.00	49.6
56.	*Chrysosporium pseudomerdarium*	GPCK 674	214.99	115.9
57.	*Chrysosporium queenslandicum*	ITCC 4731	137.66	57.8
58.	*Chrysosporium queenslandicum*	GPCK 664	196.66	110.5
59.	*Chrysosporium queenslandicum*	GPCK 665	145.99	114.2
60.	*Chrysosporium queenslandicum*	GPCK 666	159.00	112.5
61.	*Chrysosporium queenslandicum*	GPCK 667	210.66	113.6
62.	*Chrysosporium queenslandicum*	GPCK 668	217.00	113.8
63.	*Chrysosporium queenslandicum*	GPCK 669	157.99	79.4
64.	*Chrysosporium tropicum*	GPCK 644	271.33	114.8
65.	*Chrysosporium tropicum*	GPCK 645	355.66	118.5
66.	*Chrysosporium tropicum*	GPCK 646	159.99	110.5
67.	*Chrysosporium tropicum*	GPCK 647	206.33	111.2
68.	*Chrysosporium sulfurium*	GPCK 675	336.32	113.7
69.	*Chrysosporium sulfurium*	GPCK 676	224.99	119.9
70.	*Chrysosporium zonatum*	ITCC 4732	236.32	110.4
71.	*Chrysosporium zonatum*	GPCK 698	423.99	118.5

a) Mean ± S.D. = Significant at P < 0.05.
**Bhadauria and Kushwaha (2002)*

In most of the reports on constitutive keratinases, the nature of the enzyme is based on their caseinolytic rather than keratinolytic activity. It appears that keratinolytic activity is mostly inducible. Further, simple sugars such as glucose have been reported to suppress the synthesis of keratinase due to catabolic repression (Santos et al. 1996; Ignatova et al. 1999; Mohamedin, 1999; Singh, 1999; Wang and Shih, 1999; Yamamura et al. 2002 ; Bernal et al. 2003; Suntornsuk and Suntornsuk, 2003; Thys et al. 2004), which is a well known phenomenon for microbial proteases (Gupta et al. 2002). However, comparison of keratinolytic titers of various microorganisms is difficult due to the variety of substrates and the definitions of keratinase units employed. As far as physical parameters for production are concerned, they are species-specific and thus vary with respect to the organism (Williams et al. 1990; Friedrichand Antranikian, 1996; El-Naghy et al. 1998; Sangali and Brandelli, 2000; Vidal et al. 2000; Rissen and Antranikian, 2001; Rozs et al. 2001; Yamamura et al. 2002 ; Riffel et al. 2003; Thys et al. 2004). Alkaline pH from 6 to 9 supports keratinase production and feather degradation in many cases. Temperature for keratinase production ranges from 28 to 50°C for most bacteria, actinomycetes and fungi to as high as 70°C for *Thermoanaerobacter* and *Fervidobacterium* spp. (Friedrichand Antranikian, 1996; Rissen and Antranikian, 2001; Nam et al. 2002). Psychrotrophic production of keratinase has also been reported for *Stenotrophomonas* sp. (Yamamura et al. 2002). Keratinase has been produced under submerged shaking conditions, except for a few thermophilic bacteria (Friedrich and Antranikian, 1996; Nam et al. 2002; Rissen and Antranikian, 2001) and fungi (Kaul and Sumbali 1999; Singh 1999) where static submerged fermentation has been reported. Parihar and Kushwaha (2001) studied protein release from feathers during solid state fermentation.

Keratin is used as an inducer, all fermentations leading to keratinase production are also accompanied by subsequent degradation of keratin substrate. Kinetics of keratinase production and that of keratin degradation were explained by Ramnani and Gupta (2004). They also indicated that keratinolysis cannot serve as a marker for keratinase production and vice versa this is also indicated by Williams et al. 1990; Sangali and Brandelli, 2000; Vidal et al. 2000; Kim et al. 2001; Ramnani and Gupta, 2004; Thys et al. 2004), whereas keratinase degradation takes from 24 h to several days ((Ramnani and Gupta, 2004, Kaul and Sambali, 1999).

TEMPERATURE AND SUBSTRATE REQUIREMENT

The enzyme from *Chrysosporium keratinophilum* (Dozie et al. 1994) and thermophile *Fervidobacterium islandicum* (Nam et al. 2002) showed exceptionally high temperature optima of 90 and 100°C, respectively, with a half-life of 30 and 90 min, respectively. Thermal stabilization of the enzyme in the presence of divalent cations such as calcium has also been reported (Mukhopadhyay and Chandra, 1990; Dozie et al. 1994; Bressollier et al. 1999; Chitte et al. 1999; Ignatova et al. 1999; Rissen and Antranikian, 2001; Nam et al. 2002; Riffel et al. 2003; Farag and Hassan, 2004). Keratinases have a broad substrate specificity and are active against both soluble and insoluble proteinaceous substrates. Among soluble proteins, they possess the ability to hydrolyze casein, gelatin, bovine serum albumin and hemoglobin, whereas among insoluble proteins, they hydrolyze feathers, wool, silk, collagen, elastin, horn, stratum corneum, hair, azokeratin and nail. Many keratinases have not been sequenced but sequence homologies of some known keratinases indicate that they belong to subtilisin family of serine proteases.

INDUSTRIAL INVOLVEMENT OF KERATINASE

Keratinases from microorganisms have attracted a great deal of attention in the recent decade, particularly due to their multitude of industrial applications such as in the feed, fertilizer, detergent, leather and pharmaceutical industries. Currently, the most promising application of keratinases and keratinolytic microorganisms is the production of nutritious, cost-effective, environmentally benign feather meal for poultry. Besides their use in traditional industrial sectors like detergent, medicine, cosmetics, leather and feed (Farag and Hassan, 2004), they also find application in newer fields like prion degradation for treatment of the dreaded mad cow disease (Langeveld et al. 2003), biodegradable plastic manufacture and feather meal production and thus can be aptly called “modern proteases”. Although many applications of keratinases are still in the stage of infancy, a few have found way to commercialization, particularly the use of Bioresource International’s Versazyme for feather meal production (Gregg, 2002). Despite the availability of several reports on keratinolytic microorganisms, which are increasing by the day, there is only one extensive review by Onifade et al. (1998) available on keratinases that emphasizes their biotechnological potential with respect to feather meal production. Further, the mechanism of keratinolysis is highly complex and not yet well understood. Since the keratinases of many microorganisms particularly fungi have not exploited it is therefore need of the day to exploit them. Other potential uses of keratinases include the anaerobic digestion of poultry waste to generate natural gas for fuel (Brutt and Ichida, 1999), modification of fibers such as silk and wool (Rissen and Antranikian, 2001), in medicine and pharmaceuticals for elimination of acne or psoriasis,

elimination of human callus for preparation of 30 vaccines for dermatophytosis and additives in skin-lightening agents as they stimulate keratin degradation (Vignardet et al. 2001).

Table 2. Keratinase production by some soil inhabiting keratinophilic fungi

Fungus		Keratinase Ku/ml Substrate		
		Pig hair*	Peacock feather**	Chicken feather+ Human hair***
Acrodontium album	IMI179837	182.2	15	190.2
Aspergillus quercinus	IMI179848	32.2	39	60.5
Aspergillus ustus	IMI179847	47.2	55	60.4
Botryotricum keratinophilum	IMI185322	93.2	21	110.4
Chaetomium globosum	IMI179869	12.2	21	30.4
Chrysosporium crassitunicatum	IMI185320	130.2	31	160.5
Chrysosporium tropicum	IMI179840	77.2	88	92.4
Chrysosprium indicum		75.0	-	85.9
Curvularia indica	ITCC1879	132.2	33	140.4
Gliocladium agrawalii	IMI179846	118.9	88	128.8
Gliocladium rorseum	IMI179845	68.2	78	75.5
Keratinophyton terreum	IMI185313	107.5	165	118.7
Malbranhea pulchella	IMI179843	146.2	73	170.2
Microsporum fulvum	IMI194744	86.7	78	96.9
Microsporum gypseum	IMI194745	69.6	2	85.8
Penicillium lilacinum		141.2	40	158.4
Trichoderma harzianum	IMI179833	34.1	15	60.3
Trichoderma hematum	IMI185323	28.8	64	58.6
Trichophyton mentagrophytes	IMI194747	112.4	4	115.6
Verticillium lecanii	IMI185318	187.8	95	195.4

**Kushwaha and Nigam (1996)*
***Kushwaha (1983)*
****Kushwaha (2007 Unpublished).*

The new research, which tested the effects of a bacterial enzyme keratinase on brain tissues from cows and sheep with, showed that when the tissue was pretreated and in the presence of a detergent, the enzyme fully degraded the prion, rendering it undetectable. The researchers now plan another study to test the effectiveness of the enzyme on the treated prions in mice. Effectiveness in decontaminating equipment that processes animal by-products. Many scientists believe that mad cow disease is spread by healthy animals eating feed containing by-products from BSE-infected animals. Using keratinase to gobble up harmful prions on the processing equipment would go a long way in reducing the risk of spreading mad cow disease. Shih hit upon the idea of using keratinase to degrade prions based on his more than two decades of work as a poultry scientist looking for ways to manage poultry waste. He discovered that a bacteria, *Bacillus licheniformis* strain PWD-1, could degrade chicken feathers. Shih isolated and characterized the bacterial enzyme keratinase, and then isolated and sequenced the gene that encodes keratinase. He was able to develop a way to produce mass quantities of the enzyme. Shih found that keratinase can be added to chicken feed to increase digestibility and the efficiency of the feed; that is, chickens who eat feed with the enzyme grow to optimal weight quicker and need less feed to grow to that optimal weight. The enzyme thus can provide the same benefit in feed that antibiotics currently provide. Animal producers are looking for safer substitutes to antibiotics, and Shih believes that keratinase can serve that purpose.

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