A comparative characterization of indigenous keratinase enzymes from district Khairpur, Sindh, Pakistan

Pardeep Kumar, Yasmeen Faiz Kazi* and Irshad Hussain Soomro
Department of Microbiology, Shah Abdul Latif University, Khairpur, Pakistan

Abstract: To isolate and characterize keratinolytic fungi and bacteria from indigenous soils, a total of 80 samples were collected from Ghari Mori District. Khairpur, and these organisms were isolated using standard microbiological technique. The isolated keratinolytic microorganisms comprised: *Absidia* sp., *Chrysosporium asperatum, Chrysosporium keratinophilum, Entomophthora coronata, Bacillus subtilis* and *Staphylococcus aureus* and their keratinolytic properties were distinguished from the production of keratinase by measurement of zone of hydrolysis on skimmed milk agar ($P<0.05$). *C.keratinophylum* and *B. subtilis* produced largest zone among all the isolated species. The crude keratinase revealed that the optimum time for production of the enzyme was seven days, optimum temperature 30^{o}C and optimum pH 9 for *C.keratinophylum* but for *B. subtilis*, the optimum time was three days, optimum temperature 37^{o}C and optimum pH 7. The enzyme activity of *C. keratinophylum* and *B. subtilis* were determined to be 220 U/ml and 260 U/ml respectively ($P< 0.05$).

Keywords: Keratinolytic microorganism, isolation, crude enzyme, characterization.

INTRODUCTION

Keratins are the widely distributed fibrous proteins of our environment found in epithelial cells of vertebrates and characterized by its high content of amino acids, especially cystine, arginine and serine. It is present in hair, feather, hooves, wool, horns, nail, stratum and cornium (Sharma and Prashar, 1997). A vast quantities of chickens are being utilized every day in the society that produces a large amount of feathers waste in poultry industries Keratin-consisting materials have always been plentiful in the nature but restricted in practical usages, mainly because of their insolubility and non-degradability by the ordinary proteolysis, the presence of the disulfide linkages , hydrophobic interactions, and hydrogen bonds, but however, are easily digested by alkali and keratinase enzymes, a group of proteinase enzymes that have high level of activity on insoluble environmental pollution, keratin (Onifade 1998; Fuchs, 1995).

It has been studied by Jan, *et al*. (2003) that prion, an infectious agent involved in transmissible spongiform enkephalitis was degraded by keratinase produced by *B. licheniformis* strain PWD-1. They described that this enzymatic degradation could lead to the development of a method for the decontamination of medical and laboratory equipment.

The prospective use of keratinases with diverse applications might br benefited where keratins are needed to be hydrolyzed, such as medicine for degradation of keratinized skin due to psoriasis and dermatitis. In spite of all the work that has been done so far on the production of proteolytic enzymes, relatively little information is available on keratinases (Wang and Shih, 1999; Sangali and Brandelli, 2000). This is even more, the case for keratinases IN our region, Khairpur where keratinases and keratinase producing microorganisms have not been reported so far. The aim of present study was to isolate and identify keratinolytic fungi and bacteria from soils of District Khairpur and prepare crude enzyme from these species to characterize the indigenous keratinase enzymes and this is the first report elucidation of the keratinase enzyme from this region.

**Corresponding author:* yfkazi@yahoo.com

MATERIALS AND METHODS

Isolation of microorganism

A total of eighty (80) soil samples were collected randomly from different sites of Ghari Mori, District Khairpur. Samples were collected from fertile land, animal manger, poultry farms and Barbour shop. Soil samples were collected in the sterile polythene bags and brought to the Research laboratory of Department of Microbiology, Shah Abdul Latif University, Khairpur for the isolation of keratinase enzyme producing microorganisms

Isolation of Keratinase producing fungi by hair bait technique

During the present work, Hair Bait Technique was used with modification of sterilization of hair prior to placing them on the test soil.

Three replicates were used for each sample. The Petriplates were incubated at 30^{o}C. At periodic intervals (one to two weeks), the plates were examined for the development of mycelium on the hair filament Hair over-grown containing mycelium were removed with the help

of sterilized forcep and mounted in 2-3 drops of 10% KOH solution A piece of invaded hair was inoculated on surface of the Sabouraud's Dextrose Agar (SDA) and Czapek Dox Agar (CDA) in Petri plates in duplicates with help of sterile needle. Then the plates were incubated at 30°C in the incubator for 1-3 weeks. The growing individual fungal colony was transferred to fresh media.

Isolation of Keratinase Producing Bacteria from Soil

During present work, isolation of bacteria was done by soil dilution method and Spread Soil Method (Subha Rao, 2002) as follows:

Soil sample collected from fertile land and poultry farm was dried at room temperature. The soil (0.1 gm) was directly plated on melted and cooled nutrient agar medium (15 ml /plate). Before the agar solidifies the plate was rotated to distribute the soil in the medium. After solidification, plate was incubated at 37°C for 1-2 days. This procedure was done with each sample in duplicates. After incubation the plates were observed for bacterial growth, then microscopy was done by preparing Gram stained smear.

Screening for Keratinolytic Activity and Identification

The purified bacterial and fungal isolates were placed on 10% skimmed milk agar plates (prepared following the procedure of Mozammel Hoq *et al.*, 2005) and were incubated at 30 and 37°C respectively for 1-4 days. After incubation plates were observed for the zone of hydrolysis.. Depending upon the maximum relative diameter of zones, the strains were selected for further experimental studies. The isolated fungal and bacterial strains were identified on the basis of colony morphology, cultural characters, cell morphology, motility, Gram staining reaction and biochemical profiling.

Production of Keratinase

Fungus Cultivation

The indigenous fungal isolates were cultivated in Basal salt medium (table 1) and incubated by submerged fermentation in Erlenmayer flasks and incubated in rotary shaker at 30°C, 80 rmp for 1 week.

Table 1: Composition of basal salts medium

$K_2 HPO_4$	1.5g
Mg SO_4. $7H_2O$	0.025g
Ca Cl_2	0.025g
Fe SO_4. $7H_2O$	0.015g
Zn SO_4. $7H_2O$	0.005g
Water (Distilled)	1000mL
Bovine serum albumin	5 mg/mL
pH	6.8

Bacterial Cultivation

Keratinolytic bacterial strains (*Bacillus* sp) were grown on basal salt solution containing ground chicken feather powder. The chicken feathers were washed, dried and ground in grinder (Anex, Germany) prior to adding to the medium and pH was adjusted to 7.5. The medium was autoclaved, inoculated with pure culture and incubated at 37°C for 72 hours on orbital shaker (150 rpm).

The strains were identified according to standard microbiological techniques and by growing them on feather meal broth containing keratin as sole source of carbon and nitrogen.

Crude enzyme production

The cultures (Bacterial and fungal) were centrifuged at 8000 rpm, at room temperature for 10 minutes. The supernatant was collected and passed through 0.45µm pore size membrane filter (Acrodisc) and was used as crude enzyme.

Protein estimation

The concentration of soluble protein was measured by the Bradford assay according to the manufacturer's instructions (Sigma). The protein concentration was determined by using BSA as standard.

The filtrate was collected in sterile flask and assayed for proteolytic activity using azocasien assay as follows:

Azocasein protease assay

To a 50µL filtrate, 50µL L-cysteine (50 mM) was added. For control, 50µL filtrate was added to 50µL DH_2O and for blank, 50 µL DH_20 was added to 50µL basal salt solution. All reaction mixtures were incubated in duplicates at 37°C for 30 min. After incubation, 400 µl Azocasien solution (5 mg/ ml, Azocasein in 0.01M PBS pH 7) was added and incubated at 37°C for 3 h. After that 1 ml 10% TCA was added. The reaction mixture was centrifuged at 8000 rpm for 10 minutes at room temp. and to the I ml supernatant, 1ml 0.5 M NaOH was added. The absorbance was measured at $440_{nm.}$

One unit of keratinolytic activity was defined as the amount of enzyme that produces an increase of corrected A_{440nm} of 0.01 under the conditions described. The data presented are mean values of two parallel determinations.

RESULTS

Isolation of Keratinolytic fungi

The fungal strains having keratinolytic activity were isolated from soil of different sites of Khairpur district. The Hair Bait technique was applied to screen the keratinolytic fungi from the soil samples. The plates shown growth of fungi as well as bacteria on hairs (fig. 1).

Fig. 1: Growth of fungi on hair by 'Hair Bait Technique'

Microscopy of invaded hair

Further to confirm the growth of fungi, KOH mount of the invaded hair was performed. The hair showed the growth of fungi with spores and hyphae (fig. 2).

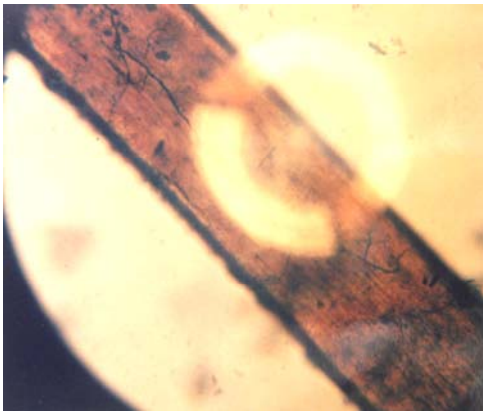

Fig. 2: Growth of fungi on surface of hair.

Isolation rate of Keratinolytic organisms

The keratinolytic fungal strains were identified on the basis of colony morphology, cultural characters, pigmentation and morphology of hyphae and spores as *Absidia sp*., *Chrysosporium asperatum*, *Chrysosporium keratinophilum* and *Entomophthora coronata*. The bacterial strains were identified on the basis of colony morphology, Gram stain reaction and biochemical characters according to the Bergey's manual of determinative bacteriology, 8th edition. Absidia sp. appeared to have highest isolation rate followed by *Chrysosporium keratinophilum, Staphylococcus aureus, Chrysosporium asperatum, B. subtilis* and lowest shown by *Entomophthora coronata* as shown in table 2.

Screening and characterization of Keratinolytic microorganisms from district Khairpur

The microorganisms isolated from the soil samples were screened by hydrolysis of skimmed milk agar. The highest zone diameter (mm) was produced by *Chrysosporium keratinophilum* followed by *B. subtilus* as shown in table 3. In order to determine the keratinolytic activity of crude enzyme, the strains of fungi and bacteria were cultivated in a submerged fermentation as described in materials and methods.

Keratinolytic activity of the culture filtrates (Crude enzyme) appeared after three days and reached its maximum after 7 days for the fungal strains and *C keratinophylum* showed highest activity (220 U/mL). On the other hand, *B. subtilis* showed maximum activity (260U/mL) at 72 hr of incubation after which the activity declined.

The effect of temperature, pH and incubation time on proteolytic activity was determined by growing keratinolytic fungi and bacteria on skimmed milk agar. The activity was assessed by measuring the zone of hydrolysis of casein in the medium.

Among the temperatures analysed (figure not shown),*Chrysosporium keratinophilum* produced maximum zone of 24mm at 30°C while other fungal strains also produced maximum zone at that temperature. So the optimum temperature for the protease activity of the fungal strains was selected to 30°C. *B. subtilis* produced maximum zone of hydrolysis at 37°C where as at the other temperatures i.e. at 45°C and 50°C, the zone of hydrolysis was smaller as compared to the temperature 37°C, but at 55°C *B. subtilis* produced only small growth on skimmed milk agar while no zone of hydrolysis was observed at 60°C. When different pHs were used for skimmed milk agar, the fungal strain showed maximum

Table 2: Isolation rate (%) of Keratinolytic microorganisms from soil of district Khairpur

Keratinolytic organisms	Fertile Land (n=20)	Animal manger (n=20)	Poultry Farms (n=20)	Barbers shop (n=20)	Total (n=80)
Absidia sp.	6 (30%)	7 (3%)	11 (55%)	2 (10%)	26 (32.5%)
Chrysosporium asperatum	3 (15%)	8 (40%)	1 (5%)	2 (10%)	14 (17.5%)
Chrysosporium keratinophilum	7 (35%)	4 (20%)	8 (40%)	1 (5%)	20 (25%)
Entomophthora coronata	2 (10%)	1 (5%)	3 (15%)	0 (0%)	6 (7.5%)
B. subtilis	6 (30%)	2 (10%)	1 (5%)	0 (0%)	9 (11.25%)
Staphylococcus aureus	1 (5%)	3 (15%)	6 (30%)	5 (25%)	15 (18.75%)

Soil samples (N=80) were collected from different sampling sites of District Khairpur. The values are mean of two separate experiments.

Table 3: Screening and Characterization of Keratinolytic microorganisms (for Fungi, 30°C and for bacteria 37°C) from District Khairpur

Keratinolytic organism	Zone diameter(mm) After 3 days of incubation	Keratinolytic activity (U/mL)	Optimum pH	Optimum temp (°C)	Optimum time (days)
Absidia sp.	6	60	9	30	7
Chrysosporium asperatum	17	160	9	30	7
Chrysosporium keratinophilum	24	220	9	30	7
Entomophthora coronata	18	100	9	30	7
B. subtilis	20	260	7.5	37	3

The values are mean of two separate experiments.

Zone Diameter

	One-Sample Statistics			
	N	Mean	Std. Deviation	Std. Error Mean
Absida sp	2	6.0000	.00000[a]	.00000
C. asperatum	2	17.0000	1.41421	1.00000
C. keratinophylum	2	24.0000	1.41421	1.00000
E. coronata	2	18.0000	1.41421	1.00000
B. subtilis	2	20.0000	1.41421	1.00000

a. t cannot be computed because the standard deviation is 0.

Zone Diameter

One-Sample t-Test						
	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
C. asperatum	17.000	1	.037	17.00000	4.2938	29.7062
C. keratinophylum	24.000	1	.027	24.00000	11.2938	36.7062
E. coronata	18.000	1	.035	18.00000	5.2938	30.7062
B. subtilis	20.000	1	.032	20.00000	7.2938	32.7062

Enzyme Activity

	One-Sample Statistics			
	N	Mean	Std. Deviation	Std. Error Mean
Absida sp	2	60.0000	1.41421	1.00000
C. asperatum	2	160.0000	1.41421	1.00000
C. keratinophylum	2	220.0000	1.41421	1.00000
E. coronata	2	100.0000	2.82843	2.00000
B. subtilis	2	260.0000	1.41421	1.00000

One-Sample t-Test						
	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Absida sp.	60.000	1	.011	60.00000	47.2938	72.7062
C. asperatum	160.000	1	.004	160.00000	147.2938	172.7062
C. keratinophylum	220.000	1	.003	220.00000	207.2938	232.7062
E. coronata	50.000	1	.013	100.00000	74.5876	125.4124
B. subtilis	260.000	1	.002	260.00000	247.2938	272.7062

hydrolysis of caseine at pH 9 while *B. subtilis* showed highest zone at pH 7.5. The optimum incubation time for production and enzyme activity for fungal strains appeared as 7 days while *B. subtilis* showed highest production and activity after 3 days of incubation. When activity of crude enzyme was determined by azocasein assay, maximum keratinolytic activity was found in fungal strain *Chrysosporium keratinophilum* and bacterial strain *B. subtilis* (table 3).

STATISTICAL ANALYSIS

Result Interpretation

Characterization was analyzed in two different replicates, taking two separate tests for zone of inhibition and keratinolytic activity. The mean difference was found through statistical procedure applying PASW Statistics 18. Test applied was t-test statistic. Various t values of five related organisms are given in four separate tables. Mean difference along with difference between two tails is also shown. The p value in both cases is < 0.05 and shows that there is a significant difference in two replicate analysis of the organisms at different levels.

DISCUSSION

Extensive work is being carried out in different areas of the world on the keratinase enzyme producing organisms. Literature survey reveals that different organisms produce keratinase enzymes such as fungi, bacteria and actinomycetes that hydrolyze the keratin protein present in skin, hairs, feathers, horns and hoofs of animals.

The aim of this study was to isolate and identify keratinolytic organisms from indigenous soils of Khairpur. For this purpose, the number and type of organisms were found to be varied in different areas from which the soil samples were collected.

During this investigation 04 species of keratinolytic fungi namely *Absidia sp. Chrysosporium asperatum, Chrysosporium keratinophilum, Entomophthora coronata* and two bacterial isolates namely *Bacillus sp. and Staphylococcus aureus* were isolated and identified from the soil of Ghari Mori, District Khairpur.

Among these 06 species of keratinolytic organisms, the *Absiedia* sp. was predominant keratinolytic fungus because it was isolated from 26 (28.9%) out of 80 soil samples collected. *Entomophthora coronata* was the lowest, isolated from 06 (6.7%) samples collected. Initial studies revealed that keratinolytic microorganisms are present in indigenous soils of Khairpur and the technique used to isolate them was improved in order to enhance the chances of isolation.

The Hair bait technique used in present study revealed successful isolation of keratinolytic fungi. The fungal strains isolated were further identified by their cultural characteristics on growth medium and by microscopy Three bacterial species were isolated namely *S.aureus, Pseudomonas sp.* and *B. subtilis* that shown keratinolytic activity however, the activity of first two was unstable at higher temperatures whereas only *B. subtilis* retained the hydrolytic activity at higher temperatures. Mozamnel *et al*. (2005) isolated *Bacillus* having keratinolytic activity from feather decomposed soil, poultry farms, and leather manufacturing industries. Other bacteria have also been reported to produce keratinase. A study on feather hydrolysis by a *Vibrio sp.* strain kr 12 has been done by Sangali and Brandelli (2000). They reported that *Vibrio sp.* strain kr 12 produced a high keratinolytic activity when cultured on native feather containing broth at optimum pH 6.0 and temperature 30^{o}C. This strain (kr12) showed activity on Azokeratin, and Azocasein. For screening of the enzyme activity, skimmed milk agar was used as a medium of choice. Maximum zone of hydrolysis was observed in *C. keratinophylum* (fungi) and *B. subtilis* at 30 and 37^{o}C respectively. When the incubation time was increased for this fungus from three to seven days, the zone of hydrolysis attained its maximum size that indicated that growth and enzyme activity of this strain require extended time to accomplish hydrolysis. The *B. subtilis* however, had shown maximum zone after three days of incubation.

Measurement of enzyme activity was performed by azocasein assay,and the cultures of fungi and bacteria were grown in basal salt medium containing keratin as source of carbon. The results revealed proteolytic activity in culture filtrates of both fungi and bacteria. It has been reported that the proteolytic activity was found to be located predominantly in the cell homogenate in *T.gallinase* while enzyme activity in *T. verrucosum* was located in the culture filtrate (Grzyzwnowicz *et al.* 1989). Ryoji *et al*. (1989) reported the keratinolytic enzyme activity from culture filtrates of *T. mentagrophytes*. An extra cellular proteinase with keratinolytic activity from strain of *Hendersonula toruloidea* was demonstrated by Vibon *et al*. (1990). El-Fadaly and Zaied (1999) reported keratin biodegradation monitored by measuring both activities of keratinases and proteinases as well as the free amino acids during the fermentation by *Bacillus* and *Micrococcus* strains culture filtrate.

The characterization studies of *C. keratinophylum* and *Bacillus* sp. were performed to investigate the optimum incubation time, temperature and pH for growth and enzyme activity of these strains. The optimum incubation time was seven days, the optimum temperature was 30^{o}C and optimum pH was 9 for *C.keratinophylum.* El-Naghy *et al* (2001) investigates that *Chrysosporium georgiae* has highest keratinolytic activity after 3 week of incubation at pH 6 and 8, 30^{o}C. *C. georgiae* degraded white chicken feather while bovine, human hair and sheep wool were not affected (El-Naghy *et al*, 2001). Muhsin and Hadi, (2002) reported highest keratinase activity by *Chrysosporium punnicola* and *M. gypseum* in the culture medium supplemented with human hair.

In present study, for *Bacillus sp.* optimum incubation time was 3 days, optimum temperature was 37^{0}C and optimum pH was 7. Mozammel, *et al*. (2005) studied the keratinolytic activity of *Bacillus* species isolated from

effluent of tannery and poultry farm using a feather enrichment technique. These strains/species were identified as *B. licheniformis, B. cereus, B. subtilis, B. brostelensis* and *B. sphericus* and most of isolated *Bacillus* species showed high levels of keratinolytic protease on feather and hair keratin in basal medium at 37^{o}C and pH 8.0.

In 1992 Xiang, *et al.* reported from the culture medium of feather degrading bacterium *Bacillus lichniformis* PWD-1 and the optimum pH 7.5, optimum temperature 50^{o}C and stable when stored at -20^{o}C. Alexandere, *et al*. (2005) investigated a novel keratinase from *B. subtilis* S14 which exhibited dehairing capabilities. They proposed that this enzyme can be an alternative to sodium sulfide and may completely replace it.

Cheng, *et al*. (2008) reported the purification and characterization of keratinase from a *Bacillus subtilis* strain KD-$N_{2.}$ and reported it's molecular weight to be 30.5 KDa, optimum pH 8.5 and optimum temperature 55^{o}C. Worapot *et al.* (2005) studied the purification and characterization of keratinase from thermo tolerant feather degrading bacterium, *Bacillus licheniformis* with optimum pH 8.5 and optimum temperature 60^{o}C.

Kaul and Sumbali (1999) investigated 14 keratinolytic fungi belonging to ten genera. They found that all the fungi grown on keratinous material released sulphydral containing compounds detected as cysteine total proteins and extracellular keratinase which was produced in maximum quantity in presence of glucose and vitamins. Daniel and Simoes (2008) characterized keratinase from a *Streptomyces* isolated from poultry plant waste water. They observed optimum keratinolytic activity at 40^{o}C and pH 8.0.

Our study largely supplements the contemporary literature and reveals that the keratinase from different microoorganisms exhibited different growth conditions for optimum production and activity of keratinase enzymes.

CONCLUSION

The fungal strain *Chrysosporium keratinophylum* and *Bacillus sublitis* produced the highest zones of hydrolysis on skimmed milk casein agar and proeolytic activity in azocasein assay. The optimum incubation time for *C. keratinophylum* was 7 days, optimum pH 9 and optimum temperature 30^{o}C. For *Bacillus subtilis*, the optimum incubation period was 3 days, optimum pH: 7 and optimum temperature: 37^{o}C.

REFERENCES

Alexandre JM, Walter OB, da Silva, Gava R, David Driemeier D, Antonio J, Henriques P and Carlos Termignoni C (2005). Novel keratinase from *Bacillus subtilis* S14 exhibiting remarkable dehairing capabilities. *App. Env. Microb.,* **71**(1): 594-596.

Cheng SW, Hu HM, Shen SW, Takagi H, Asano M and Tsai YC. (1995). Production and characterization of keratinase of a feather-degrading *Bacillus licheniformis* PWD-1. *Biosci. Biotech. Biochem.,* **59**: 2239-2243.

Cheng-gang C,Chen JS, Qi J, Yin Y and Zheng X. (2008). Purification and characterization of keratinase from a new *Bacillus subtilis* strain. *J. Zhej. Uni. Sci.,* **9**(9): 713-720.

Daniel MTT and Simoes MLG. (2008). Production and partial characterization of keratinase produced by a microorganism isolated from poultry processing plant wastewater. *Afr. J. Biotech.,* **7**(3): 296-300.

El-Fadaly H and Zaied KA. (1999). Microbial degradation of native keratin in batch fermentation. *Pak. J. Biol. Sci.,* **2**(3): 627-634.

El-Naghy MA, el-Ktatny MS, Fadl Allah EM and Nazeer WW (2001). Degradation of chicken feathers by *Chrysosporium georgiae*. *Mycopathologia.,* **143**(2): 77-84.

Fuchs E. (1995). Keratins and the skin. *Annu. Rev. Cell Dev.***,** 11**:** 123-153.

Grzywnowicz, Lobarzewski J, Wawrzkiewicz K and Wolski T. (1989). Comparative characterization of proteolytic enzymes from *Trichophyton gallinase* and *Trichophyton verrucosum. J. Med. Vet. Mycol.,* **27**: 319-328.

Jan PM L, Dick FM Van de WG, Jan J and Alex Bossers A. (2003). Enzymatic degradation of prion protein in brain stem from infected cattle and sheep. *J. Infect. Dis.,* **188**: 1782-1789.

Kaul S and Sumbali. (1999). Production of extra cellular keratinases by keratinolytic fungal species inhabiting feathers of living poultry birds (*Gallus domesticus*). A comparison. *Mycopathologia.,* **146**(1): 19-24.

Mozammel Hoq, Siddiquee K, Kawasahi H and Tatsuji Seki T. (2005). Keratinolytic activity of some newly isolated Bacillus species. *J. Biol. Sci.,* **5**(2): 193-200.

Muhsin TM and Hadi RB. (2002). Degradation of keratin substrates by fungi isolated from sewage sludge. *Mycopathologia.,* **154**(4): 185-189.

Subha R NS. (2000). Soil microbiology, soil microorganisms and plant growth, 4th Edition IBH Published by Raju primlani for Oxford and IBH CO. 66,Janpath, New Delhi, India, 65.

Onifade AA. (1998). A review: Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. *Biores. Tech.,* **66**: 1-11.

Ryoji R, Ko I, Takamori K and Ogawa H. 1989 Isolation of a keratinolytic proteinase from *Trichophyton mentagrophytes* with enzymatic activity at acidic pH. *Infect. Immun.,* **57**(11): 3479-3483.

Sangali S and Brandelli A (2000). Feather keratin hydrolysis by a vibrio species. Strain kr2. *J. App. Microb*., **89**(5): 735-743.

Sharma JL and Parshar RK (1997). A Dictionary of Biochemistry. CBS Publisher and Distributors, Dehli, India, 74.

Vibon R, Yoshiike T,Tsuboi R, Takamori K and Ogawa H. (1990). Purification and characterization of an extra-cellular proteinase from *Hendersonula toruloidea*. *Infect. Immun*., **58**: 2856-2861.

Wang JJ, Shih JCH, Jch Shih (1999). Fermentation production of keratinase from *Bacillus licheniformis* PWD-1 and a recombinant *B. subtilis* FDB-29. *J. Indus Microb. Biotech.,* **22**(6): 608-616.

Worapot S, Tongjun J, Onnim P, Oyama H, Rutanakanokchai K, Kusamran T and Oda K. (2005). Purification and characterization of keratinase from a thermotolerant feather degrading bacterium. *W. J. Microb. Biotech.,* **21**: 1111-1117.

Xiang L,Lee CG, Casale ES and Shih JC. (1992). Purification and characterization of a keratinase from a feather degrading *Bacillus licheniformis* strain. *App. Env. Microb.,* **58**(10): 3271-3275.