INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 10, p. 104-115, 2015

RESEARCH PAPER OPEN ACCESS

Keratinolytic activity of *Cladosporium* and *Trichoderma* species isolated from barbers' landfill

Nwadiaro Patience, Ogbonna Abigail*, Wuyep Ponchang, Adekojo Deborah

Department of Plant Science and Technology, University of Jos, Nigeria

Key words: Fungi, Keratinolytic, Human hair, Skim milk, Chicken feather.

http://dx.doi.org/10.12692/ijb/6.10.104-115 Article published on May 30, 2015

Abstract

Studies were carried out on the ability of filamentous fungi to degrade keratin based substrates (human hair and chicken feather) using their keratinase. Soil samples were collected at random from barbers' landfills in Jos city, Nigeria and were screened using the hair baiting technique. Proteolytic ability of the fungal isolates was carried out using skim milk agar. Fungal strains that showed the highest activity following the diameter zones of clearance were further tested for keratinase activity in submerged fermentation (SmF) using basal mineral medium supplemented with human hair and chicken feather as sole sources of carbon and nitrogen. A total of 17 fungal species belonging to 10 genera were isolated with *Aspergillus* species the most dominant. *Cladosporium cladosporioides* and *Trichoderma viride* had the highest potential to hydrolyze skim milk casein with the peak period of 72h (77.1U/ml and 50.1U/ml) for *C. cladosporioides* and *T. viride*, respectively, after which there was a decline in enzyme production. The keratinase activity of the two species on both human hair and chicken feather were highly variable. *C. cladosporioides* and *T. viride* had keratinase activity of 6.3 U/ml and 37.5 U/ml after 72h and 96h, respectively, on human hair while for chicken feather medium, *C. cladosporioides* had keratinase activity of 35.5 U/ml after 96h and *T. viride* 37.5 U/ml after 72h. The spent medium containing chicken feather showed higher specific activity for keratinase as compared to the spent human hair medium. It is deduced that these fungi may have an important role in the degradation of keratin containing wastes in a natural environment.

* **Corresponding Author:** Ogbonna Abigail ✉ ogbonabi@yahoo.co.uk

Introduction

Keratinized materials such as Hairs, feathers, hoof, furs, claws, nails, and horns are produced in huge amounts annually as wastes products in various human and animal processing industries (Poole *et al.*, 2010; Brandelli *et al.*, 2010). Keratins generally have a high degree of cross-linkages by disulfide and other bonds and are known as insoluble protein. Keratins are of two types- α-Keratins which are present in wool, hair, and horn and β-Keratins which are present in feather in the form of polypeptide chain. These materials are known for their strong stability against chemical reagents such as acids, alkalis and are resistant to degradation to many proteases including papain, pepsin and trypsin but are easily digested by keratinase enzymes (Mabrouk, 2008). Keratinases are enzymes that hydrolyze keratin containing substrates specifically. Keratinolytic enzymes may have potential roles in biotechnological processes involving keratin containing wastes from various industries that produce keratinized waste materials.

Keratin utilization has been reported in variety of both aquatic and terrestrial fungi. Keratinophilic fungi are small, well defined and important group of fungi that colonize various keratinous substrates and degrade them to components of low molecular weight (Najwa and Jamel, 2013). These fungi are present in the environment with variable distribution patterns which depend on factors, such as human and or animal presence. These species of keratinophilic fungi depending on their natural habitat are anthrophilic when human beings become their natural hosts, zoophilic when animals act as their natural hosts and geophilic when they inhabit the soil. Soil has proved to be the natural reservoir of these fungi (Ritesh *et al.*, 2013). Majority of the keratinophilic fungi found in soil are not dermatophytes but soil inhabitants and are therefore fast growing nonpathogenic keratinophilic fungi. Keratin decomposition in soil leads to an increase in carbon and nitrogen ratio in soil which helps in the mineralization of soil for increased nutrients.

The present study was carried out with the view to obtain keratinolytic proteases with high ability to degrade the keratinous wastes, especially human hair and chicken feathers. Also the keratinases so obtained may be exploited for their biotechnological potential in industries.

Materials and methods

Materials

Soil samples were collected from three locations of barbers' landfills where the cut human hairs are emptied. The soil samples were collected from topmost part of the soils (5cm depth) with sterile hand trowel and were collected inside sterile polyethylene bags (well labeled). The soil samples were transported to the laboratory for further analysis.

The human hairs were obtained from a barbers' landfill while the white chicken feathers were obtained from a poultry farm Jos, Nigeria. Both the human hairs and the chicken feathers were washed thoroughly in three changes of distilled water. They air-dried and were defatted by soaking in diethyl ether for 24 hours. They were thoroughly washed again with distilled water and were completely dried in hot air oven at 60^{o}C for 24 hours, ground into powder with sterilized grinding stone. The chicken feathers were ground without the midrib. The pulverized human hair and chicken feathers were used in the preparation of mineral media used for the determination of keratinolytic activity of the isolated fungi.

Isolation of keratinophylic fungi

The modified hair baiting method of Vanbreuseghem, 1952 was used for the isolation of keratinophylic fungi. A weight of 50g of each soil sample was transferred to a sterile Petri plate, and pieces of sterilized and deffated human hair were spread over the soil in the plate. The hair-baited soils in the plates were moistened with sterile distilled water and incubated at room temperature (25±2°C) for 28 days. The soil samples were periodically moistened with small quantities of sterile distilled water to prevent dryness. The soil samples were examined routinely for fungal growths on the hair baits. Portions of the

growth were aseptically transferred to already prepared Petri plates of Sabouraud Dextrose Agar (SDA) supplemented with 2mg/ml gentamycin. The inoculated plates were incubated at room temperature for five to fifteen days. The fungal growths were sub-cultured severally in order to obtain pure cultures.

The same experiment was repeated using defatted chicken feathers. All experiments were carried out in triplicates.

Identification of the fungal isolates

Identification of the isolates done by examination of the nature of colonies on agar plates and microscopic examination of mycelia, its arrangement and the nature of the fruiting bodies. Lactophenol in cotton (LB) blue was used as mounting fluid. References were made using schemes of Domsch, *et al.* (1980), Samson, *et al.* (1984), Dubey and Maheshwari, (2007) as atlas for comparison.

Physico-chemical parameters analyses of the soil samples

Determination of soil pH, moisture and organic matter contents

The soil pH was measured by mixing the soil with water in the ratio of 1:5 and was determined by Jenway digital pH meter model 3310.

For the moisture content determination, a weight of 30 grams of soil from each soil sample was dried to a constant weight in hot air oven set at 110°C. The percentage moisture contents of the soil samples were determined in triplicates.

For the organic matter content determination, soil samples (30 grams) previously dried to a constant weight in hot air oven set at 110°C were used in the determination of the percentage (%) organic matter content. The soil samples were put in porcelain crucibles and the crucibles were placed in a muffle furnace and heated at 400°C for 3hours. The samples were cooled and the percentage organic content of the soil samples was determined in triplicates and then recorded.

The concentrations of calcium (Ca), Potassium (K), magnesium (Mg) and Sodium (Na) were recorded as mg/k of metal using atomic absorption spectrophotometer (BUCK 210 VGP). Nitrogen in the soil samples was determined by Kjeldahl method. The percentage nitrogen was then determined by distillation using 40% NaOH and 4% boric acid. It was then titrated against 0.01N HCl (Olayiwola *et al.*, 2012).

Screening of fungal isolates for proteolytic activity using skim milk agar medium

One percent (1%) skim milk agar plates were prepared and inoculated with 5mm mycelia discs from the edge of actively growing 4-day old MEA cultures of the test fungi. After five days of incubation at 25±2°C, the plates were observed for clear zones of hydrolysis around the inoculated culture (Gonzalez *et al.*, 2003). Mean diameters of three replicates were recorded for each fungal species. Depending upon the maximum relative diameter of clear zones, *Cladosporium* sp. and *Trichoderma* sp. were selected for further studies.

Statistical analysis

Results are given as mean ± Standard error (SE) of N observations taken in three replicates (n = 3).

Fungal cultivation for crude protease and keratinase production using submerged fermentation (SmF)

Fungal cultivation for crude protease and keratinase production was done using modified method of Wawrzkrewicz *et al.*, (1987). Submerged fermentation (SmF) was performed by inoculating disc of pure cultures of actively growing *Cladosporium* sp. and *Trichoderma* sp. into the production medium, NaCl (0.5), KH_2PO_4 (1.5), K_2HPO_4 (1.0), $MgSO_4.7H_2O$ (0.2) containing skim milk powder (1%) as a sole source of carbon and nitrogen, in two separate Erlenmeyer flasks (250ml). This same procedure was carried out with production medium supplemented with ground human hair (1%) and ground chicken feather (1%) used as keratin sources respectively. The

Erlenmeyer flasks were incubated for fifteen days at room temperature (27±2°C) without agitation. The supernatant after the mycelia was filtered off served as the crude enzyme source. The supernatant was assayed for extracellular proteases (skim milk casein) and keratinases (human hair and chicken feather).

Assay of keratinolytic protease

Determination of keratinolytic activity using skim milk casein as substrate

The reaction mixture contained 1ml of crude enzyme, 1ml of phosphate buffer, pH 7.0, 1 ml of 1% skim milk casein and 1 ml of 10mM $CaCl_2$ (Al-Sane *et al.* 2002). The reaction tubes were incubated at different temperatures of 37°C for 30mins. The reaction was stopped by placing the tubes in an ice bath, followed by filteration after cooling to remove the substrate. The supernatant was spectrophotometrically measured at 520nm. One unit of protease activity was defined as an increase in absorbency of 0.01 at 520 nm under the standard assay conditions.

Determination of keratinolytic activity using human hair and chicken feather as substrates

The assay was done according to modified method of Gradisar *et al.* (2000). The reaction mixture contained 1ml of crude enzyme, 1ml of 1% ground human hair (substrate) and 1 ml of Glycine-NaOH buffer, pH 9.0. The reaction tubes were incubated at different temperatures of 37°C for 1hr and the reaction was stopped by the addition of 1 ml of Dintrosalicylic Acid (DNS), followed by filteration after cooling to remove the substrate. The supernatant was spectrophotometrically measured at 595nm. One unit of keratinase activity was defined as an increase in absorbency of 0.01 at 595 nm under the standard assay conditions.

Effect of pH on enzyme activity

The effect of pH on the keratinolytic activity was determined using skim milk casein, ground human hair and chicken feather as substrates using the modified method of Thoomatti and Peramachi (2012). Keratinolytic activity was studied in the pH range of 3 to 10 using the following buffers. 0.2 M sodium acetate buffer, pH (3 to 5), 0.2 M phosphate buffer (6 to 8) and 0.2 M Tris-HCl buffer (pH 9 to 10).

Effect of temperature on enzyme activity

The effect of different temperatures on enzyme activity was studied using modified method of Thoomatti and Peramachi (2012). The optimum temperature for keratinolytic protease activity was determined by performing the enzyme reaction at incubation temperatures between 25°C and 60° C.

Results

The identification of the fungal isolates resulted into 17 species belonging to 10 genera of fungi (Table 1). Among the identified species, 5 belonged to *Aspergillus*, representing 29.41%, 2 belonged to *Penicillium*, representing 11.76%, 1 belonged to *Basidiobolus, Cladosporium, Emericella, Eupenicillium, Mucor, Rhizopus, Trichoderma, Trichophyton*, representing 5.88% each. Also 1 species of sterile fungal isolate and unidentified fungus was made, representing 5.88%. Of the three locations studied, locations B and C recorded 13 species of fungi while location A recorded 11 species of fungi. The genus *Aspergillus* had five species including *Aspergillus flavus, A. niger, A. fumigatus, A. terreus* and *A. unilateralis*, 2 species of *Penicillium* included *P. chrysogenum* and *P.* sp, one species each of *Basidiobolus* sp, *Cladosporium cladosporioides, Emericella nidulans, Eupenicillium* sp, *Mucor* sp, *Rhizopus sp, Trichoderma viride, Trichophyton mentagrophytes,* mycelia sterilia and an unidentified sp. The species of *Trichophyton mentagrophyte* was the only dermatophyte isolated from the soil samples. Other species of the fungal isolates were non-dermatophytes and were saprophytic in nature (Table 1). The species that were most frequently isolated included *Aspergillus flavus, A. niger, A. fumigatus A. terreus, Cladosporium cladosporioides* and *Trichoderma viride* (100%). This was followed by *Basidiobolus* sp, *Emericella nidulans, Eupenicillium* sp, *Mucor sp, Penicillium chrysogenum, P.sp and Rhizopus sp* (66.7%). This was followed by *A. unilateralis,* mycelia sterilia and unidentified sp (33.3%).

Table 1. Fungal species isolated from experimental soil samples.

S/No	Fungal Isolates	Locations			Total
		A	B	C	C
	Aspergillus flavus Link	+*	+ *	+	3
2	*A. niger* van Tieghem	+	+	+	3
3	*A. fumigatus* Fresenius	+	+	+	3
4	*A. terreus* Thom	+	+	+	3
5	*A. unilateralis*	-	+	-	1
6	*Basidiobolus* sp	-	+	+	2
7	*Cladosporium cladosporioides* (Fresen.) de Vries	+	+	+	3
8	*Emericella nidulans* (Eidam) Vuillemin	+	-	+	2
9	*Eupenicillium* sp	+	+	-	2
10	Mycelia sterilia	-	-	+	1
11	*Mucor* sp	+	+	-	2
12	*Penicillium chrysogenum*	-	+	+	2
13	*Penicillium* sp	+	-	+	2
14	*Rhizopus sp*	-	+	+	2
15	*Trichoderma viride*	+	+	+	3
16	*Trichophyton mentagrophytes*	+	-	+	2
17	Unidentified sp	-	+	-	1
	Total	11	13	13	37
	% frequency of occurrence	73.3	86.7	86.7	

+=present - =absent.

Ecological and Elemental analysis of the soil samples

The ecological parameters determination revealed that the pH values of the soil samples A, B and C were 5.95, 6.04 and 6.46, respectively. From these results, it shows that the three soil samples are acidic in nature but soil sample A was more acidic than samples B and C. The results of the pH of the experimental soil samples are presented in Table 2.

Table 2. Ecological and Elemental Parameter of the Experimental Soil samples.

Parameter	Soil Sample A	Soil Sample B	Soil Sample C
pH	5.95	6.04	6.46
Moisture content %	10.78	15.55	12.08
Nitrogen %	0.095	0.11	0.010
Organic Matter %	3.28	3.62	0.34
Phosphorus mg/kg	2.1	45.5	1.4
Sodium mg/kg	1.3	1.4	2.0
Potassium mg/kg	28	100	20
Calcium mg/kg	880	1080	900
Magnesium mg/kg	72	108	80

The moisture content of the experimental soil samples revealed the values of 10.78% for sample A, 15.55% for sample B and 12.08% for sample C, respectively. Also, the organic content of the soil samples revealed the values of 3.28% for soil sample A, 3.62% for soil sample B, and 0.34% for soil sample C. The details of the results are shown in Table 2.

The elemental analysis of the experimental soil samples revealed the Nitrogen content to be 0.095%, for soil sample A, 0.11% for soil sample B, and 0.010% for soil sample C respectively. The other elements that were assessed included Phosphorus (2.1, 45.5, 1.4mg/kg), Sodium (1.3, 1.4, 2.0mg/kg), Potassium (28, 100,20mg/kg), Calcium (880, 1080, 900mg/kg)

and Magnesium (72, 108, 80mg/kg) for soil samples A, B and C respectively. The details of the result of elemental analysis are presented in Table 2.

Determination of proteolytic activity using skim milk agar medium

The proteolytic activity of the test organisms using skim milk as sole source of carbon and nitrogen showed that about 50% (6) of the fungal isolates were proteolytic while *A. niger*, *A. unilateralis, Eupenicillum* sp, *Basidiobolus* sp, *A. flavus* and *A. fumigatus* initiated growth but did not hydrolyze the skim milk. There was no any zone of hydrolysis shown by them. The results also showed that *Cladosporium cladosporioides* had the highest diameters of clear zone of 60mm. *Trichophyton mentagrophytes* and *Trichoderma viride* showed 50 and 55mm respectively. The diameter zones of hydrolysis measured for *Emericella nidulans* and *Aspergillus terreus* are 40 and 45mm respectively. The least diameter zone of hydrolysis of 35mm was recorded for *Penicillium* sp. *Cladosporium cladosporioides* and *Trichoderma viride* showed maximum growth and activity and were therefore selected for further studies. The results of determination of proteolytic activity of the test organisms using skim milk agar medium are presented in Table 3 and Plate 1.

Table 3. Proteolytic activity of the fungal isolates, using skim milk agar.

Fungal Isolates	Growth On Skim Milk agar	Clear zone	Diameter zone (mm)
Aspergillus terreus	++	+	45 ±1.7
Cladosporium cladosporioides	+++	+	60±2.0
Penicillium sp.	++	+	35±1.0
Trichophyton mentagrophytes	++	+	50±1.0
Trichoderma viride	+++	+	55±1.0
Emericella nidulans	++	+	40±2.7
A. niger	+	-	-
A. unilateralis	+	-	-
Basidiobolus sp.	++	-	-
Eupenicillum sp.	++	-	-
A. flavus	+	-	-
A. fumigatus	+		

Each data represents the mean of three replicates (standard error)

The symbols +++, ++, +, - indicates high, moderate, low, and no activity respectively.

Assay of keratinolytic enzyme produced by the fungal isolates

Determination of protease activity using 1%skim milk powder as substrate

The culture filtrates from *Cladosporium cladosporioides* and *Trichoderma viride*, grown on skim milk medium were tested for their protease activities. It was found that the spent medium containing skim milk showed higher specific activity for protease. The result revealed that harvesting time of 72hrs was ideal for maximum protease production for both species. The protease activity increased with increase in time until the peak period of 72hrs (77.1U/ml) after which there was a decline in enzyme production. The results of the protease activity using 1% skim milk powder as substrate is presented in Fig. 1.

Determination of keratinolytic activity using 1% human hair and chicken feather as substrates

The culture filtrates from *Cladosporium cladosporioides* and *Trichoderma viride*, grown on human hair and feather media were tested for their keratinase activities. It was found that the keratinase production by the two species was highly variable.

For the human hair medium, *C. cladosporioides* had its maximum keratinase production at 72hrs (6.3U/ml) while *T. viride* had its maximum production at 96hrs (37.5U/ml), while for the chicken feather medium, *C. cladosporioides* had its maximum keratinase production at 96hrs (35.5U/ml) and *T. viride* at 72hrs (37.5U/ml). It was found that the spent medium containing chicken feather showed higher specific activity for keratinase as compared to the spent human hair medium (Fig. 2a and 2b). Highest specific activities were found on the fourth and third day of growth of *T. viride* in spent media with human hair and feather respectively. In all, both media supported the growth of *T. viride* better than *C. cladosporioides*. The results of keratinolytic activity using 1% human hair and chicken feather as substrates are shown in fig. 2a and 2b.

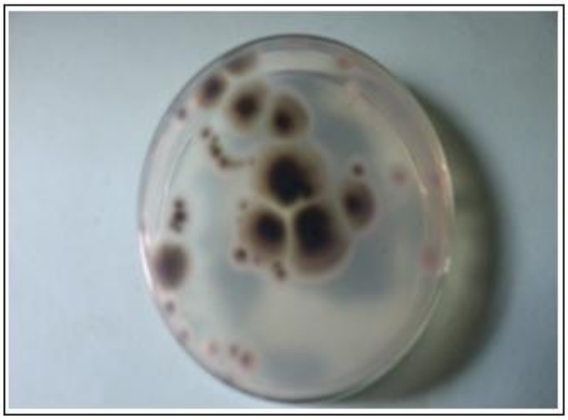

Plate 1a. *Cladosporium cladosporioides* showing clear zone on skim milk agar after 7days of incubation

Effects of different pH on Enzyme activity

The effect of pH on the activity of keratinase in the range of 3.0 -9.0 showed a gradual increase in keratinase activity with increase in pH and the maximum activity was at pH 8 for both *Cladosporium cladosporioides* and *Trichoderma viride* on skim milk, human hair and chicken feather media. The results of the effect of pH on the activity of keratinase are presented in fig. 3a and 3b.

Effects of temperature on enzyme activity

The effects of temperature on enzyme activity as indicated in Tables 4a-4c showed that for both skim milk, human hair and chicken feather media, the enzymes showed optimal activity at 50°C (155U/ml), and 37°C (27.5U/ml) for *Cladosporium cladosporioides* and *Trichoderma viride*, respectively for skim milk medium. For human hair medium the enzyme activity was at 50°C (20.3 U/ml) and 37°C (29.2 U/ml) for *C. cladosporioides* and *T. viride* respectively. For chicken feather medium the enzyme activity was at 50°C (14.9 U/ml) and 37°C (38.3 U/ml) for *C. cladosporioides* and *T. viride*, respectively. It was observed that further increase in temperature resulted in decline in the enzyme activity.

Plate 1b. *Trichoderma viride* showing clear zone on skim milk agar after 7 days of incubation.

Discussion

Screening of soil samples from barbers' dumping ground revealed a total of 17 isolates belonging to 10 genera *Aspergillus, Basidiobolus, Cladosporium, Emericella, Eupenicillium, Mucor, Penicillium, Rhizopus, Trichoderma* and *Trichophyton* were isolated and assessed for their keratinolytic ability. One species of sterile fungus and one unidentified fungus were also isolated (Table 1). The genus *Aspergillus* was the most prevalent, of which *A. niger* was the dominant species. Although some of the fungi isolated are known soil fungi, the presence of keratin in the soil could promote their distribution and growth (Jeong-Dong, 2003). Some of these isolates were previously thought as non-keratinolyilic but studies have shown that these fungi possess keratinolytic activity when grown on keratin-rich substrates and they also occur in the environment in keratin-rich soil (Soomro and Zardari, 2002; Soomro *et al.*, 2006). Most of the fungi isolated from this study are present in soils of different areas (Sarquis, 1990). *Aspergillus* species, *Penicillium* species and *Cladosporium* species are distributed worldwide (Abu

et al., 2004). *Trichophyton mentagrophytes* isolated from this study is a well-known zoophilic dermatophyte and has been frequently recovered from soils in some countries (Ogbonna and Pugh, 1987; Deshmukh and Verekar, 2006).

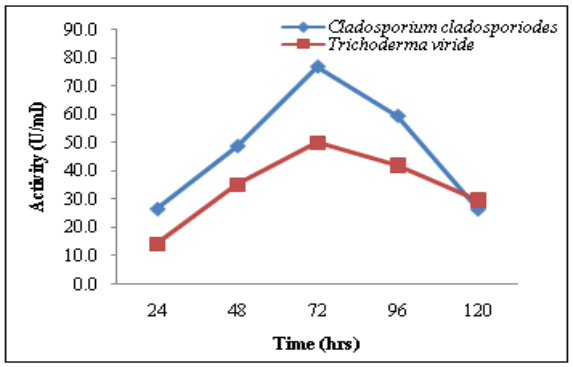

Fig. 1. Protease activity using 1%skim milk powder as substrate.

The soil sample A recorded the pH value of 5.95 while that of soil sample B and C were found to be 6.04 and 6.46, respectively. This indicates that the soil sample A is slightly acidic while samples B and C are slightly alkaline. The pH values recorded for soil samples were within the ranges that support microbial growth in culture and other microbial activities.

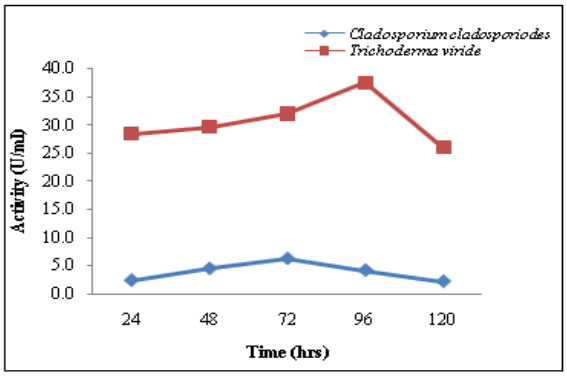

Fig. 2a. keratinase activity using 1% human hair as substrate.

The moisture content of the soil samples were recorded as 10.78%, 15.55% and 12.08% for samples A, B and C, respectively. These ranges of moisture content values also support microbial growth and other activities including humus production.

The organic matter (OM) content of the soil samples as shown in Table 2 indicates that the soil samples A and B have organic matter contents of 3.28% and 3.62% respectively, while the least value of 0.34% organic matter (OM) content was recorded for soil sample C. However, the increase in the number of isolates in location C could be attributed to other factors such as pH and water content that played significant roles in activities of the microorganisms in natural environments such as soil. This shows that microbial load in the soils, diversity and their metabolic activities is fully supported by the site conditions especially the soil pH and there is therefore an adequate production of OM. It is reported that slightly acidic and alkaline soils like the ones obtained in this work, promote biodegradation activity of microorganisms in soils (Obire and Nwaubeta, 2002).

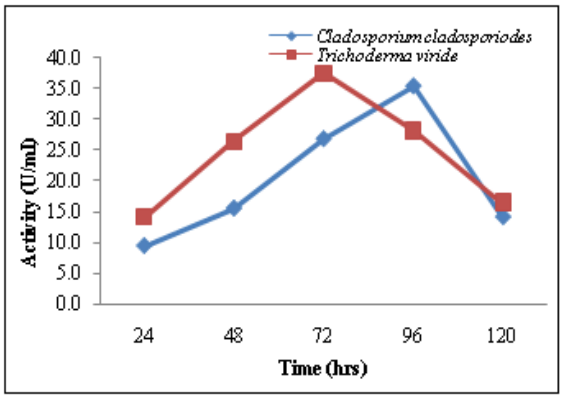

Fig. 2b. keratinase activity using 1% chicken feather as substrate.

Screening tests on skim milk agar showed that the isolates were able to grow on the agar medium (Table 3). All the fungal isolates exhibited growth on the milk agar but not all developed clear zones. The organisms that developed clearing zones on the milk agar secreted hydrolytic enzymes and showed protease activity. The zone of hydrolysis was measured at 5 days of incubation. About 16.6% of the isolates showed 60mm zone of clearing which was the highest zone. This highest zone was produced by *Cladosporium cladosporioides*. About 33.3% of the fungi showed 50-55mm zone of clearance and included *Trichophyton mentagrophytes* (50mm) and *Trichoderma viride* (55mm). Also 33.3% of the fungi showed 40-45%and include E*mericella nidulans* and *Aspergillus terreus*. 16.6% of the isolates showed 35 mm of clear zone produced *Penicillium* sp at the 5th day of incubation. The other fungi that grew but did

not show any zone of hydrolysis included *Aspergillus niger, A. unilateralis, Basidiobolus sp, Eupenicillium sp, A. flavus and A. fumigatus*. These fungi did not hydrolyze the medium probably due to lack of extracellular enzymes. All the fungi that hydrolyzed the skim milk agar showed maximum protease activity. It was based on these results that *Cladosporium cladosporioides* and *Trichoderma viride* were selected for the production and assay of proteases and keratinase (enzymes) in submerged fermentation.

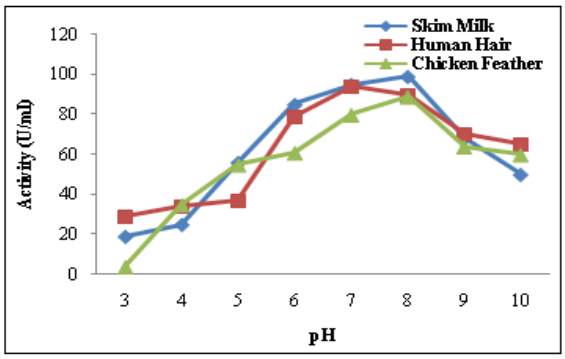

Fig. 3a. Effect of pH on enzyme activity of *Cladosporium cladosporioides*.

From the study, it was observed that the only one species of dermatophyte, *Trichophyton mentagrophytes* isolated could not hydrolyze the milk agar as much as the other species of hyphomycetes (*Cladosporium cladosporioides* and *Trichoderma viride*). Prerna and Kushwaha (2011) had reported maximum keratinolytic activity in *Cladosporium* sp, *Chrysosporium indicum* and *Acremonium chrysogenum*.

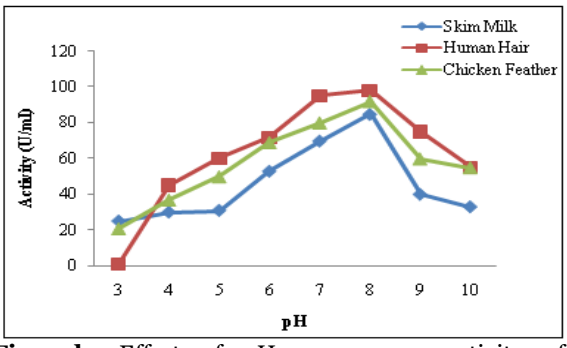

Fig. 3b. Effect of pH on enzyme activity of *Trichoderma* viride.

The culture filtrates of the two selected fungal species showed protease/keratinase activity on mineral medium supplemented with skim milk, human hair and chicken feather in submerged fermentation (SmF). Keratinous wastes are insoluble proteins and microbial keratinolysis is a protein degrading proteolytic process. These two fungal species were able to hydrolyze the keratinous materials used in the study showing that they had the ability to degrade the insoluble proteins. Kanchana, (2013) reported that the capability of filamentous fungi to degrade keratinous materials may be as a result of a combination of extracellular keratinase and sulphitolysis (reduction of disulphide bonds by sulphite excreted by mycelia) which may allow other less specific protease to act, resulting in an extensive keratin hydrolysis. Keratin degradation takes from 24 h to several days. This is probably attributed to the complex mechanism of keratinolysis of these fungi.

The keratinases produced from the three different media (skim milk, human hair and feather) were found to be active with a broad pH range, with the optimum pH of 8 for both *Cladosporium cladosporioides* and *Trichoderma viride*. This result is in agreement with that of Radhika *et al*., (2013). Most keratinases have been reported to be active in neutral to alkaline conditions, pH 7-9 (Thys *et al*., 2004; El-Refai, 2005), few of extreme alkalophilic optima, pH 12- 13 (Mitsuiki, 2004).

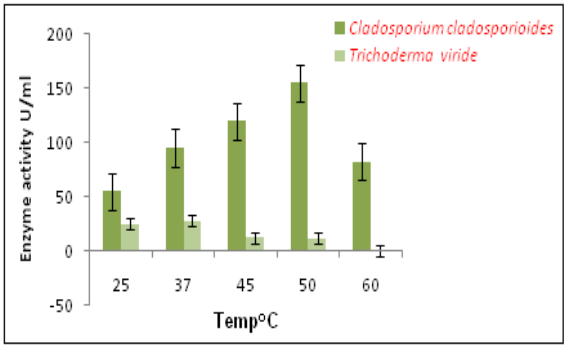

Fig. 4a. Effect of Temperature on Enzyme Activity using Skim Milk (U/ml).

The effect of different temperature regimes on the activity of the keratinases indicated that the optimum temperature was 50°C for *Cladosporium cladosporioides* and 37°C for *Trichoderma viride* from both sources (skim milk, human hair and feather media). These results show thermophilic and thermotolerant nature of the enzymes (Fig. 5a-5c).

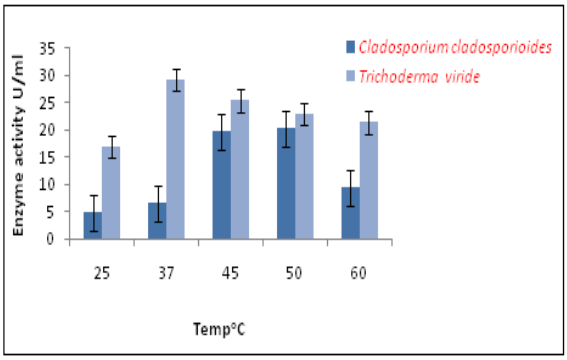

Fig. 4b. Effect of Temperature on Enzyme activity using Human Hair (U/ml).

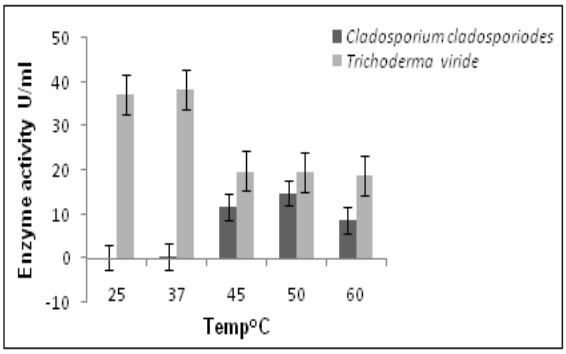

Fig. 4c. Effect of Temperature on Enzyme activity using Chicken feather (U/ml).

Thoomatti and Peramachi (2012) reported a similar result in their work on production and characterization of keratinolytic proteases from *Aspergillus parasiticus*. However, reports including those of Gradisar *et al.*, (2000); Cao *et al.*, (2008); Kanchana, (2013) indicated that keratinolytic proteases have thermophilic and thermotolerant optimum temperatures between 35- 55°C.

Conclusion

Fungal species were isolated from soil samples collected from human hair dump site in Jos city, Nigeria using hair baiting technique. The fungal species were tested for proteolytic activity using skim milk agar. *C. cladosporioides* and *T. viride* were found to be highly proteolytic and were used for the keratinase enzyme assay. The keratinase activity of the two species on both human hair and chicken feather were highly variable. *C. cladosporioides* and *T. viride* had keratinase activity of 6.3 U/ml and 37.5 U/ml after 72h and 96h, respectively, on human hair while for chicken feather medium, *C. cladosporioides* had keratinase activity of 35.5 U/ml after 96h and *T. viride* 37.5 U/ml after 72h. The spent medium containing chicken feather showed higher specific activity for keratinase as compared to the spent human hair medium. It is then deduced that these two species have the biotechnological potential for the degradation of complex keratinous substrates in natural setting.

References

Abu P, Hilda A, Gopinath SC. 2004. Keratinophilic fungi of poultry farm and feather dumping soil in Tami Nadu, India. Mycopathologia **158(3),** 303-909. http://link.springer.com/article/10.1007%2Fs11046-004-3465-1

Al-Sane NA, Al-Musallam AA, Onifade AA. 2002. The isolation of keratin degrading microorganisms from Kuwait soil: production and characterization of their keratinases. Kuwait Journal of Science and Engineering **29(2),** 125-138.

Brandelli A, Daroit DJ, Riffel A. 2010. Biochemical features of microbial keratinases and their production and applications. Applied Microbiology and Biotechnology **85,** 1735-1750. http://dx.doi.org/10.1007/s00253-009-2398-5.

Cao L, Tan H, Liu Y, Xue X, Zhou S. 2008. Characterization of a new keratinolytic *Trichoderma atroviride* strain F6 that completely degrades native chicken feather. Letters in Applied Microbiology **46,** 389-394. http://dx.doi.org/10.1111/j.1472-765X.2008.02327.x

Deshmukh SK, Verekar SA. 2006. The occurrence of dermatophytes other keratinophilic fungi from the soils of Himachal Pradesh (India). Czech Mycology **58,** 117-124.

Domsch KH, Gams W, Anderson TH. 1993. Compendium of Soil Fungi. In: Domsch, K.H., Gams, W., Anderson, T.H. *Eching, Vol.* ***1,*** *Germany: IHW-Verlag.*

Dubey RC, Maheshwari DK. 2007. A manual of practical Microbiology. Chand and company limited, Ramnagar New Delhi 1- 397.

El-Refai HA, AbdelNaby MA, Gaballa A, El-Araby MH, AbdelFattah AF. 2005. Improvement of the newly isolated *Bacillus pumilus* FH9 keratinolytic activity. Process Biochemistry, **40(7),** 2325-2332. http://dx.doi.org/10.1016/j.procbio.2004.09.006.

González VG, Favela-Torres E, Aguilar CN, Rómero-Gomez, S. D´ıaz-God´ınez G, Augur C. 2003. Advantages of fungal enzyme production in solid state over liquid fermentation systems. Biochemical Engineering Journal **13,** 157-167.

Gradisar H, Kern S, Friedrich J. 2000. keratinases of *Deratomyces microspores*. Applied microbiology and Biotechnology **53,** 196-200.

Jeong-Dong K. 2003. Keratinolytic Activity of Five *Aspergillus* Species Isolatedfrom Poultry Farming Soil in Korea. Mycobiology, **31(3),** 157-161. http://dx.doi.org/10.4489/MYCO.2003.31.3.157

Kanchana R. 2013.Utilization of biodegradable keratin containing wastes by enzymatic treatment. International Journal of Pharmacy and biological Sciences **4(1),** (B) 117 -126.

Mabrouk MEM. 2008. Feather degradation by a new keratinolytic *Streptomyces* sp. MS-2. World Journal of Microbiology and Biotechnology **24,** 2331-2338.

Mitsuiki S, Ichikawa M, Oka T, Sakai M, Moriyama Y, Sameshima Y, Goto M, Furukawa K. 2004. Molecular characterization of a keratinolytic enzyme from an alkaliphilic *Nocardiopsis* sp. TOA-1 Enzyme. Microbial Technology **34(5),** 482-489.

Najwa M, Jameel A, Abu M. 2013 Biochemical study for three keratine baiting and using it to isolation and identification some fungi from soil in Basrah, Iraq. International Journal of Engineering and Innovative Technology (IJEIT) **3(3),** 216- 225.

Obire O, Nwaubeta O. 2002. Effects of refined petroleum hydrocarbon on soilphysicochemical and bacteriological characteristics. Journal of Applied Sciences and Environmental Management **6(1),** 39-44.

Ogbonna CIC, Pugh GJF. 1987. Keratinophilic fungi from Nigerian soil. *Mycopathologia*. **99(2),** 115-118.

Olayiwola OA, Latona DF, Oyeleke GO. 2012. Evaluation of the macronutrients composition of soil, leaves and seeds of African yam bean (*Sphenostylis sternocarpa* harms) IOSR. Journal of Applied Chemistry **1(1),** 13-17.

Poole AJ, Church JS, Huson MG. 2009. Environmentally sustainable fibers from regenerated protein. Biomacromolecules **10,** 1-8.

Prerna A, Kushwaha RKS. 2011. Keratinase activity of some hyphomycetous fungi from dropped off chicken feathers. International Journal of Pharmaceutical and Biological Archives **2(6),** 1745-1750.

Radhika SM, Riddhi JJ, Chaya SS. 2013. Optimization of cultural conditions for extracellular keratinase production by B*acillus* species isolated from poultry farm soil. International Journal of Pharmacy and Biological Sciences **4(2),** (B) 454 – 463.

Ritesh K, Rajshree M, Sudarshan M, Sahu HB. 2013. Isolation and identification of keratinophilic fungi from garbage waste soils of Jharkhand region of India. European Journal of Experimental Biology, **3(3),** 600-604.

Samson RA, Hoekstra ES, Vanoorschoot CAN. 1984. *Introduction to food-Borne Fungi*. Publ.

Centraalbureau Voorschimmelcultures Baarn, Delft Inst. of the royal Netherlands Academy of Arts and Sciences 249 p.

Sarquis MIM, Oliveira PC. 1990. Diversity of micro fungi in the sandy soil of Panema Beach, Rio de Janeiro, Brasil. Journal of Basic Microbiology **36,** 51-58.

Soomro IH, Zardari M. 2002. Investigation keratinophilic fungi from saline soil in Khairpur, Sindh, Pakistan. Hamdard Medicus **46,** 82- 85.

Soomro IH, Zardari M, Kazi YF, Shahani NK. 2006. Hair bait technique: A method for isolation of keratinase producing microorganisms from soil. Hamdard Medicus **49(1),** 49-52.

Thoomatti SA, Peramachi P. 2012. Production and characterization of keratinolytic protease(s) from the fungus, *Aspergillus parasiticus*. International journal of Research in Biological Sciences **2(2),** 87-93.

Thys RCS, Lucas FS, Riffel A, Heeb P, Brandelli A. 2004. Characterization of a protease of a feather-degrading *Mycobacterium* species. Letters in Applied microbiology **39(2),** 181-186. http://dx.doi.org/10.1111/j.1472-765X.2004.01558.x

Vanbreuseghem R. 1952. Technique biologique pour l'isolement des dermatophytes du sol. In Annales de la Societe belge de medecine tropicale **32,** 175-8.

Wawrzkrewicz K, Wolski T. Lbarzewski J. 1991. Screening the keratinolytic activity of drematophytes *invitro*. Mycopathologia **114,** 1-8.