British Microbiology Research Journal
8(1): 358-366, 2015, Article no.BMRJ.2015.128
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

Keratin Degradation by *Penicillium purpurogenum* Isolated from Tannery Soils in Jos, Nigeria

P. O. Nwadiaro[1], A. Chuku[2], I. A. Onyimba[3], A. I. Ogbonna[1*], I. A. Nwaukwu[1] and D. A. Adekojo[1]

[1]Departmentof Plant Science and Technology, University of Jos, P.M.B.2084 Jos, Nigeria.
[2]Department of Microbiology, Federal University Lafia, P.M.B.146 Lafia, Nasarawa State, Nigeria.
[3]Departmnt of Science Laboratory Technology, University of Jos, P.M.B.2084, Jos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author PON initiated and designed the study. Authors AC and IAO performed the statistical analysis. Authors PON and AIN wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors AIN, DAA and IAO managed the analyses of the study and literature searches. Authors PON, AC and IAO read the first draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/16339
Editor(s):
(1) Ram Kumar Pundir, Ambala College of Engineering and Applied Research, India.
(2) Abd El-Latif Hesham, Department of Genetics, Assiut University, Egypt.
Reviewers:
(1) Miroslawa Prochon, Department of Chemistry, Institute of Polymer and Dye Technology, Lodz University of Technology, Poland.
(2) Sunil Kumar Deshmukh, Nano Biotechnology Centre, The Energy and Resources Institute, India.
(3) Ali Mohamed Elshafei, Department of Microbial Chemistry, National Research Centre, Egypt.
(4) Anonymous, Canada.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1087&id=8&aid=8817

Original Research Article

Received 26th January 2015
Accepted 1st April 2015
Published 15th April 2015

ABSTRACT

Background: Tannery soils are very rich in keratinous materials such as fur, wool and hair that are by-products of the tanning industry where hides and skins are processed. A keratinase producing fungus belonging to the genus *Penicillium* was isolated from soils containing tannery wastes in Jos, Plateau State Nigeria.
Materials and Methods: The fungus produced extra cellular protease on skim milk casein agar as an indication for keratinase production. The species had maximum proteolytic and keratinase activities in a Submerged Fermentation (SmF) using liquid basal medium supplemented with skim milk, chicken feathers and human hair as carbon and nitrogen sources.
Results: The Spectrophotometric assay of the proteolytic and keratinolytic activity of *Penicillium*

**Corresponding author: E-mail: ogbonabi@yahoo.co.uk;*

purpurogenum, showed that it had the highest activity (13.5 U/ml) on chicken feathers. This was followed by human hair with activity of 12 U/ml. The least activity of 11.9 U/ml was observed in skim milk medium. It was observed that incubation temperature had an effect on the enzyme activity, with an optimum temperature of 37°C for both protease and keratinase.

Conclusion: This non-dermatophytic keratinolytic fungus may have potential use in biotechnological processes involving keratin hydrolysis. The results of this work reiterated that keratinolytic activity is relatively widespread among common fungi and may have an important role in keratin degradation in the natural environment.

Keywords: Skim milk; keratinolytic activity; Penicillium; chicken feather; basal medium.

1. INTRODUCTION

Soil that is rich in keratinous materials is conducive to the growth of keratinophilic microorganisms such as fungi. Keratin is the most abundant and highly stable animal protein on earth [1], therefore, keratinophilic species are ecologically important microorganisms. The keratinolytic activity of many microorganisms has been studied. Among the fungi, keratinases of dermatophytic fungi have been well documented [2]. Keratinophilic fungal communities include saprophytes, some of which are considered to be pathogenic to humans and other organisms. They occur both in soils and wastewater habitats [2]. Keratinophilic fungi decompose α-keratins, the insoluble fibrous protein. The tight packing of hair polypeptide chains in the α-helix structures and their linkages by disulphide bonds, make them poorly biodegradable [3].

Tannery soils are very rich in keratinous materials such as fur, wooland hair. Prior to the tanning process, the skins of the animals are depilated and the hair piled in dumpsites. These hairs constitute environmental hazards since they accumulate in the immediate environments within the tannery [4]. Most of the tanneries in Jos city, Nigeria are located in the midst of densely populated residential areas. This may expose the inhabitants of these areas to pathogenic fungi and other microorganisms, as well as the stench associated with tanneries and other toxic chemicals accompanying the tannery wastes. These wastes may find their way into underground water sources thereby posing serious health risks to the residents. The fungal flora of Nigerian soils have been studied extensively [5-7], including studies on keratinophilic fungal species [8-10]. The keratinases of non-dermatophytic fungal species inhabiting soil have also been studied [9,10]. However, there is a dearth of information on the keratinases of waste water fungi in Nigeria. The studies on keratinophilic fungi from soils of tannery wastes in developing countries like Nigeria have been scanty. Therefore, this research work was designed to explore sources of keratinase producing fungi from soils of tannery waste dumping sites in Jos, Plateau State Nigeria which may help in the degradation of complex keratinous waste materials of man and animals in nature, thereby helping in reducing pollution.

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples

Soil sample collection was carried out using the method described by [11]. 500 g of soil contaminated with tannery wastes was collected from two different locations within the tannery site located at Naraguta Village, in Jos Nigeria. An additional sample was collected from soil devoid of tannery activities to act as a control. At each location, soil samples were collected from 3 different points making a total of 9 samples including control. All soil samples collected using a sterile hand shovel were placed in labeled sterile polyethene bags, and immediately transported to the laboratory for processing.

2.2 Collection and Processing of Human Hair and Chicken Feathers

Human hair was obtained from a barber's landfill and chicken feathers were obtained from a poultry farm. Both the human hair and chicken feathers were thoroughly washed, defatted by soaking in a solution of diethyl ether for 24 hours and then rinsed in three changes of sterile distilled water. They were completely dried (individually) in a hot air oven and ground with a sterilized grinding stone. The pulverized human hair and chicken feathers were packaged and kept for future use.

2.3 Isolation of Keratinophilic Fungi from the Soil Samples

The hair baiting method of [12] was used for the isolation of keratinophylic fungi. 50 g of each soil sample was transferred to a sterile Petri plate and pieces of sterilized and defatted 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±2ºC) for 28 days. The soil samples were periodically moistened with a small quantity of sterile distilled water. The soil samples were checked daily for fungal growth on the hair baits and cultures aseptically transferred to Sabouraud Dextrose Agar (SDA) plates supplemented with 2 mg/ml gentamycin. The inoculated plates were incubated at room temperature for five to fifteen days. The plates were examined every day for fungal growth and sub-cultured to get pure cultures.

Methods were repeated using defatted chicken feathers. All experiments were carried out in triplicates.

2.4 Identification of Fungal Isolates

Identification of isolates was done by macro and microscopic examination of mycelia and fruiting bodies with reference to the literature [13,14]. Lactophenol in cotton blue (LB) was used as mounting fluid.

2.5 Screening of Fungal Isolates for Proteolytic Activity Using Skim Milk Agar Medium

The method of [15] was used to determine the proteolytic activity of the test fungi. 1% skim milk agar plates were prepared and inoculated with 5mm mycelia discs from the edge of actively growing 4-day old 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. Mean diameters of three replicates were recorded for each fungal species. The fungus that had the highest diameter of zone of clearance was selected for further work.

2.6 Fungal Cultivation for Crude Protease and Keratinase Production Using Liquid Basal Medium

Fungal cultivation for crude protease and keratinase production was done using the methods of [16]. Submerged Fermentation (SmF) was performed by inoculating discs of pure cultures of actively growing *Penicillium purpurogenum* from Malt Extract Agar (MEA) plates into sterile production medium. The 100ml production medium contained the following per litre (gL^{-1}) of distilled water in 250 ml Erlenmeyer flasks: skim milk powder (1%) as a sole source of carbon and nitrogen, NaCl (0.5), KH_2PO_4 (1.5), K_2HPO_4 (1.0), $MgSO_4.7H_2O$ (0.2). This same procedure was carried out with production medium supplemented with ground human hair (1%) and ground chicken feathers (1%) used as keratin sources respectively. The 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). Changes in the pH of the medium were also carried out before and after the assay.

2.7 Assay of Keratinolytic Protease

2.7.1 Determination of proteolytic activity using skim milk casein as substrate

The method of [17] was employed for the assay. The reaction mixture contained 1 ml of crude enzyme, 1ml of 0.05 M phosphate buffer, pH 7.0, 1 ml of 1% skim milk casein and 1 ml of 10 mM $CaCl_2$. The reaction tubes were incubated at 37°C for 30mins. The reaction was stopped by placing the tubes in an ice bath, followed by filtration after cooling to remove the substrate. The supernatant was spectrophotometrically measured at 280 nm. One unit of protease activity was defined as an increase in absorbance of 0.01 at 280 nm under standard assay conditions. The pH of the supernatant in each tube was checked to determine the alkalinity.

2.7.2 Determination of keratinolytic activity using human hair and chicken feathers as substrates

The assay was done according to method of [18]. The reaction mixture contained 1ml of crude enzyme, 1 ml of 1% ground human hair (substrate) and 1 ml of 0.05 M Glycine-NaOH buffer, pH 9.0. The reaction tubes were incubated at 37°C for 1hr and the reaction was stopped by placing the tubes in an ice bath, followed by filtration after cooling to remove the substrate. The supernatant was

spectrophotometrically measured at 520 nm. One unit of keratinase activity was defined as an increase in absorbance of 0.01 at 520 nm under standard assay conditions. The pH of the supernatant in each tube was checked to determine the alkalinity.

2.8 Effect of Temperature on Enzyme Activity

The effect of different temperatures on enzyme activity was studied using the method of [19].The optimum temperature for keratinolytic protease activity was determined by performing the enzyme reaction at incubation temperatures between 25 and 60°C.

2.9 Statistical Analysis

Results are given as mean±Standard Deviation (SD) of N observations taken in three replicates (n = 3). Data sets were examined by one-way analysis of variance (ANOVA) using Statistical package for Social Sciences (SPSS) to compare the means of the different variables. P-value of less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1 Isolation of Keratinophylic Fungi from Soil Samples

Seven fungal species belonging to six genera were isolated from the soil samples collected from Jos tannery. Two of the genera belong to the class Phycomycetes and included *Mucor* sp and *Rhizopus* sp. The other genera belong to the class Hyphomycetes and included *Aspergillus*, *Cladosporium, Fusarium* and *Penicillium* (Table 1).

This is very similar to Maghazy [20] in his study on air keratinophilic fungi in primary schools in Assiut, Egypt which isolated *Aspergillus*, *Penicillium*, *Cladosporium*, *Chrysosporium* and *Alternaria*. Similar results were also obtained by researchers in other countries [21-24], and in Nigeria [25,10].

Table 1a. Distribution of keratinophilic fungi isolated from soils of two tanneries in Jos metropolis

Fungal species	Naraguta tannery soil		Control soil		Total	
	n	%	n	%	n	%
Phycomycetes						
Cunninghamella sp	00	00	12	4.6	12	2.7
Mucor sp	05	2.6	23	8.8	28	6.2
Rhizopus sp	09	4.8	06	2.3	15	3.3
Hyphomycetes						
Aspergillus candidus Link	00	00	09	3.4	09	2.0
A. flavus Link	40	21.2	30	11.4	70	15.5
A. fumigates Fres	00	00	12	4.6	12	2.7
A. niger van Tieghem	00	00	35	13.3	35	7.7
A. parasiticus speare	24	12.7	00	00	24	5.3
Cephaliophora irregularis Thaxt.	00	00	04	1.5	04	0.9
Cladosporium cladosporioides (Fres) de Vries	46	24.3	21	8.0	67	14.8
C. sp	00	00	10	3.8	10	2.2
Curvularia sp	00	00	11	4.2	11	2.4
Emericella nidulans (Eidam) Vuill	00	00	02	0.8	02	0.4
Fusarium solani (Mart) Sacc.	12	6.4	03	1.1	15	3.3
Paecilomyces variotii Bain	00	00	18	6.8	18	4.0
Papulospora irregularis Hotson	00	00	03	1.1	03	0.7
Penicillium citrinum Thom	00	00	13	4.9	13	2.9
Penicillium purpurogenum Stoll	53	28.0	41	15.6	94	20.8
Trichoderma viride Pers. Ex. Gray	00	00	10	3.8	10	2.2
Total	189		263		452	100
pH	7.53		6.46			

The isolation of fewer keratinophilic fungi from tannery soils compared to previous studies in other soils could be a result of the pH of the soil samples which was slightly alkaline 7.53. The pH of the control soil was 6.46 and harboured more (eighteen) fungal species (Table 1a). Soil pH is a factor that influences the distribution of fungi in any habitat. The distribution of keratinophilic fungi is influenced by soil pH and climatic factors [26]. Muhammed, [27] in a study on the distribution of geophilic dermatophytes in Kenyan soils, reported that the majority of the isolates of dermatophytes including *M. gypseum* were recovered from soils with acidic pH.

The alkaline pH of the soil samples in this study could be attributed to the tanning activities. Tannery waste water contains some proteins released during enzymatic processing of leather as well as salts and chromium (Cr), that might affect soil microbial processes and distribution [28]. The high salt content of the tannery waste could have affected the diversity of fungi in such soils as it is only halotolerant (salt loving) species that will thrive.

Eighteen fungal species belonging to thirteen genera were isolated from the control soil sample as shown in Table 1a. There was no significant difference (P>0.05) in the number of fungal species between the control soil (263) and the tannery waste contaminated soil (189) samples (Table 1b). This result is in conformity with the study of Rabah and Ibrahim [25] on physico-chemical and microbiological characterization of soils laden with tannery effluents in Sokoto, Nigeria which isolated few number of fungal species from the waste contaminated soil but differ with the study of Alvarez-Bernal et al. [28] on effect of tanneries waste water on chemical and biological soil characteristics which found the microbial biomass to be much higher in tannery waste contaminated soil than the other soil samples tested.

3.2 Proteolytic Activity of the Fungal Isolates Using Skim Milk Agar Medium

All fungal isolates were screened for hydrolytic enzyme activity on skim milk casein agar plates. Of the seven fungal strains tested, only four were proteolytic with hydrolytic zones on skim milk casein agar plates (Fig. 1). They included *Aspergillus flavus*, *Aspergillus parasiticus, Cladosporium cladosporioides* and *Penicillium purpurogenum* with hydrolytic zone diameters of 54±1.0 mm, 40±1.0 mm, 57±1.0 mm and 64±1.0 mm respectively. It was observed that *Mucor* sp and *Rhizopus* sp initiated growth on the casein agar medium but did not show hydrolytic zones on the medium. This is probably due to a lack of extracellular enzyme production. These findings agree with Awasthi and Kushwaha [9] where *Aspergillus, Alternaria, Cladosporium*, and other species isolated showed maximum keratinolytic activity on skim milk. *Fusarium* and the Mucorales including *Mucor* sp, *Rhizopus* sp and *Absidia* sp were thought of as non-keratinolytic but recent studies have shown that these groups of fungi have keratinolytic activities, especially when grown on substrates that are rich in keratin [29,30]. *Penicillium purpurogenum* had the highest diameter zone of clearance (64 mm) and was selected for further studies on enzyme assay.

3.3 Keratinolytic Activity of the Test Fungus Using 1% Skim Milk, Human Hair and Chicken Feathers

The keratinolytic activity of the cell free supernatant from *Penicillium purpurogenum* used to degrade the three substrates, 1% skim milk, human hair and chicken feathers at 37ºC, showed that there was enzyme activity on proteinous and keratinous substrates used as sole sources of carbon and nitrogen (Fig. 2). The highest activity of 13.5 U/ml was observed in chicken feathers, followed by human hair with activity of 12.0 U/ml. Skim milk agar had the least activity of 11.9 U/ml. This result is an indication that *P. purpurogenum* degraded the chicken feather and human hair better than the skim milk. It is interesting to note that this fungus has the potential to biodegrade keratin in natural setting. The insoluble non degradable chicken feathers were hydrolysed by the keratinase of this fungus. This result is similar to that of Marcondes et al. [31] in their work on new feather degrading filamentous fungi. *Aspergillus* and *Penicillium* species were among the genera of fungi isolated which were able to grow and produce keratinase in cultures using poultry feather powder as their sole source of carbon and nitrogen. The biotechnological use of microbial keratinases to upgrade the nutritional value of feather meal has been reported [32]. The pH of the medium was found to have turned alkaline and is believed to be as a result of the release of cysteine, keratinase and proteins by the fungus. It has been suggested that the basis of keratinolysis is due to high level of deamination and eventual alkalization of the medium, though deamination and alkalization surely play a serious role in keratinolysis [33-35].

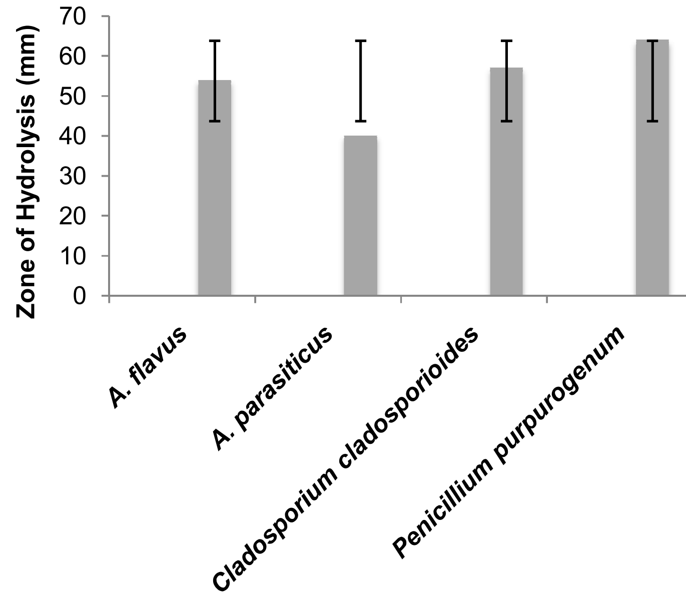

Fig. 1. Proteolytic activity of the fungal isolates using skim milk agar medium data are mean±SD(n=3)

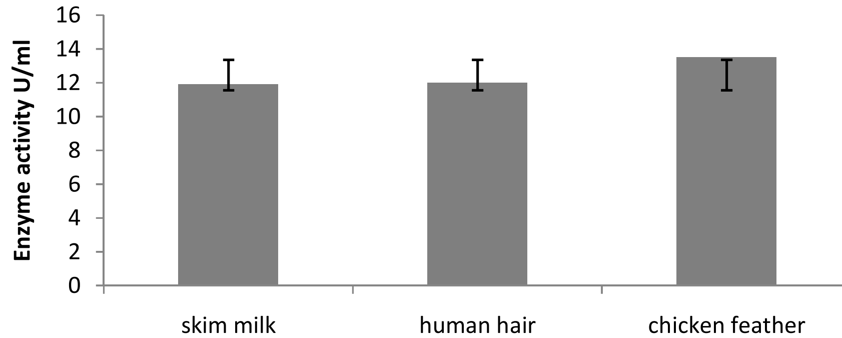

Fig. 2. Keratinolytic activity of *Penicillium purpurogenum*on 1% skim milk, human hair, and chicken feathers used as substrates data are mean±SD (n=3)

Table 1b. One way ANOVA of fungal isolates from tannery soil and control soil

			Mean	df	Fcal	Sig.
Group	**Tannery soil**	19	9.95			
	Control soil	19	13.8	36	0.660	0.422

One way analysis of variance was employed and the output indicated that there is no significant difference between the fungal isolates from the tannery soil and the control soil P>0.05

3.4 Effect of Incubation Temperature on Enzyme Activity

The effect of incubation temperature on enzyme activity as presented in Fig. 3 showed that the optimum temperature for both the protease and keratinase enzymes was 37°C. There was a statistically significant difference (P≤0.05) in enzyme activity of the fungus between the skim milk and the keratin substrates (human hair and chicken feathers). The skim milk produced an activity of 45 U/ml, while human hair and chicken feather had an activity of 88 U/ml and 87 U/ml respectively.

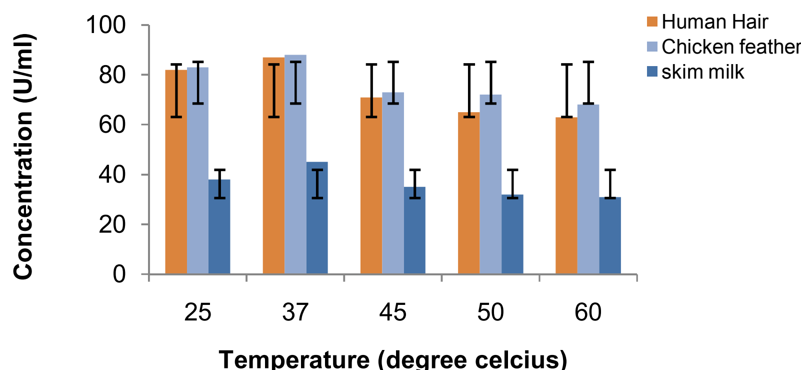

Fig. 3. Effect of incubation temperature on enzyme activity. Data are mean±SD (n=3)

The proteolytic activity increased at lower temperatures and declined at higher temperatures. The enzymes showed optimal activity and maximum degradation at 37°C which indicates they are thermotolerant in nature (Fig. 3). An increase in temperature above 37°C or decrease in temperature below 37°C resulted in a decrease in enzyme activity. Similar results have been reported in protease activity in soils treated with diary shed effluents [36] and diary factory effluents [37] where enzyme activity increased at first and then decreased with time/ temperature. The results are also in line with those obtained for keratinase production by *Penicillium* sp and other farm soil fungi [38].Incubation temperature profoundly affects the duration of enzyme synthesis phase and enzyme production [39].

The findings of this research work have added to the different types of products obtained from *P. purpurogenum.* This speciesis known for production of different kinds of industrial enzymes. Reddy and Rathod [40] reported gallic acid and tannase production in *P. purpurogenum*. Other reports included that of Steiner et al. [41] on cellulase production, Dhake and Patil [42] on β-glucosidase production and Geweely [43] on pigment production. These arrays of industrial products by *P. purpurogenum* could be useful in industries including pharmaceutical, food, textile and cosmetics.

4. CONCLUSION

Penicillium purpurogenum bio-degraded skim milk casein, human hair and chicken feather in Submerged Fermentation (SmF). It has the greatest activity on chicken feathers indicating that it could be employed in waste and environmental pollution management. In Nigeria, poultry feathers, animal hair and other keratin sources including tannery wastes do not find suitable applications. Practical use of keratinase producing microorganisms such as the one used in this study is being explored in applied microbiology where there is great need for active degraders for the management of keratin containing wastes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mercantini R, Marsella R, Caprilli F, Dovgiallo G. Isolation of dermatophytes and correlated species from the soil of public gardens and parks in Rome. Sabouraudia. 1980;18:123-128.
2. Narula N. and Sareen S. Effect of Natural Antifungals on Keratinophilic fungi Isolated from soil. Journal of Soil Science. 2011; 1(1):12-15.
3. Kunert J, Kushwaha RKS, Guarro J. Biology of dermatophytes and other keratinophilic fungi Revista Iberoamericana De Micologia Bilbao. 2000;77-85.
4. Tudunwada IY, Essiet EU, Muhammad SG. The effects of tannery sludge on heavy metals concentration in cereals on small-holder farms in Kano, Nigeria. J. Environ. Cont. 2007;35:65-69.
5. Ogunmwonyi IN, Igbinosa OE, Aiyegoro OA, Odjadjare EE. Microbial analysis of different top soil samples of selected site in Obafemi Awolowo University, Nigeria.

Scientific Research and Essay. 2008; 3(3):120-124.
6. Makut MD, Owolewa OA. Antibiotic-Producing fungi present in the soil environment of Keffi Metropolis, Nasarawa State, Nigeria. Trakia Journal of Sciences. 2011;9(2):33-39.
7. Ewekeye T, Oke O, Li-Hammed M. Soil mycoflora studies of some locations in Lagos State, Nigeria. Report and Opinion. 2012;4(4):52-57.
8. Sharma R, Rajak RC. Keratinophilic Fungi: Nature Degrading Machines! Their Isolation, Identification and Ecological Role. Resonance. 2003;13:28-40.
9. Awasthi P, Kushwaha RKS. Keratinase Activity of Some Hyphomycetous Fungi from Dropped Off Chicken Feathers. International Journal of Pharmaceutical and Biological. 2011;2(6):1745-1750.
10. Ugoh SC, Ijigbade B. Production And Characterisation of Keratinase By Fungi Isolated From Soil Samples At Gwagwalada, FCT-Abuja, Nigeria. Nat Sci. 2013;11(10):1-7. Available:http://www.sciencepub.net/nature
11. Orji MU, Nwokolo SO, Okolo I. Effect of Palm oil mill effluent on soil microflora. Nigerian Journal of Microbiology. 2006; 20(2):1026-1031.
12. Vanbreuseghem R. Technique biologique pour L'isolement des dermatophytes dusol Annalaes de la societe belge de médicine tropicale. 1952;32:175-178.
13. Domsch KH, Gams H, Anderson TH. Compendium of soil fungi. Academic press, London. 1980;859.
14. Samson RA, Hoekstra ES, Van Oorschoot CAN. Introduction to Food-Borne Fungi. Publ. Central bureau Voor schimmel cultures of the royal Netherlands Academy of Arts and Sciences. 1984;249.
15. Kanchana R. Utilization of Biodegradable Keratin Containing Wastes By Enzymatic treatment. International Journal of Pharmacological and Biological Sciences. 2013;4(1,B):117-126.
16. Wawarzkiewicz K, Lobarzewski J, Wolski T. Intracellular Keratinase of *Trichophyton gallinae* Medical Mycology. 1987;25(4): 261-268.
17. Al-Sane NA, Al-Musallam AA, Onifade AA. The isolation of keratin degrading microorganisms from Kuwait soil: production and characterization of their keratinases. Kuwait J. Sci. Eng. 2002; 29(2):12-138.
18. Ramnani P, Gupta R. Optimization of medium composition for keratinase production on feather by *Bacillus licheniformis* using statistical methods involving response surface methodology. Biotechnology Applied Biochemistry. 2004; 40:191-196.
19. Tork S, Aly MM, Nawar, L. Biochemical and Molecular Characterization of a New Local Keratinase Producing *Pseudomonas* sp. Asian Journal of Biotechnology. 2010; 2(1):1-13.
20. Maghazy SMN. Keratinophilic fungi of the air and the floor dust in primary schools in Assiut, Egypt. Minia Sci. Bull. 1989;2:121-134.
21. Harish CG. Non dermatophytic filamentous keratinophilic fungi and their role in human infection. Rev Iberoam Micología Apdo. 2000;699.
22. Kannan P, Janaki C, Selvi GS. Prevalence of dermatophytes and other fungi isolated from clinical samples. Ind. J. Med. Microbiol. 2006;24(3):212-215.
23. Soomro IH, Kazi YF, Zardari M, Shar AH. Isolation of Keratinophilic Fungi from Soil in Khairpur City, Sindh, Pakistan. Bangladesh J Microbiol. 2007;24(1):79-80.
24. Yasser MM. Keratinophilic fungi inhabiting student house at Benisuef University, Egypt. Archives of Biomedical Sciences. 2013;1(2):20-30.
25. Rabah AB, Ibrahim ML. Physico-chemical and microbiological characterization of soils laden with tannery effluents in Sokoto, Nigeria. Nigerian Journal of Basic and Applied Science. 2010;18(1):65-71.
26. Gugnani HC, Sharma S, Wright K. A preliminary study on the occurrence of keratinophilic fungi in soils of Jamaica. Rev. Inst. Med. Trop. 2014;56(3):231-4.
27. Muhammed SI, Lalji N. The distribution of geophilic dermatophytes in Kenyan soils. Mycopathologia. 1978;63:95-7.
28. Alvarez-Bernal D, Contreras-Ramos SM, Trujilo-Tapia V, Olade-portugal V, Frais-Hernandex JT, Dendooven L. Effect of tanneries waste water on chemical and biological soil characteristics. Appl. Soil Ecol. 2006;33:269-277.
29. Mercantini RM, Carvallari MC. Keratinophilic fungi isolated from Antartic soil. Mycopathologia. 1989;106(1):47-52.
30. Soomro IH, Zardari M. Investigation keratinophilic fungi from saline soil in Khairpur, Sindh, Pakistan. Hamdard Medicus. 2002;46:82-85.

31. Marcondes NR, Taira CL, Vandresen DC, Svidzinski TIE, Kadowaki MK, Peralta RM. New feather-degrading filamentous fungi. Microb. Ecol. 2008;56:13-17.
32. Shih JCH. Biodegradation and utilization of feather keratin. In proceeding of Animal Waste Management Symposium Raleigh, North Carolina State University. 1999;165-171.
33. Yu RJ, Hormon SR Blank F. Isolation, purification of an extracellular keratinase of *Trichophyton mentagrophytes*. J. Bacteriol. 1968;96:1435-1436.
34. Deshmukh SK, Agrawal SC. Degradation of human hair by some dermatophytes and other keratinophilic fungi. Mykosen. 1985; 28:463–466.
35. Kunert J. Keratin decomposition by dermatophytes. I. Sulfite production as a possible way of substrate denaturation. Z. Allg. Mikrobiol.1973;13:489-498.
36. Zaman M, Di KCC. A field study of gross rate of mineralization and nitrification and their relationships to microbial biomass and enzyme activities in soils treated with daily effluents and ammonium fertilizer. Soil Use manage. 1999;15:188-194.
37. Nizamuddin S, Sridevi A, Narasimha G. Impact of diary factory effluents on soil enzyme activities. Eco. Environ. Cons. 2008;4:89-94.
38. Agrawal D, Patidar P, Banerjee T, Patil S. Production of alkaline protease by *Penicillium*sp. under SSF conditions and its application to soy protein hydrolysis. Process Biochemistry. 2004;39:977-981.
39. Suresh PV, Chandrasekaran M. Impact of process parameters on chitinase production by an alkalophilic marine *Beauveria bassiana* in solid state fermentation. Process Biochemistry. 1999; 34:25-267.
40. Reddy SB, Rathod V. Gallic acid production and tannase activity of *P. purpurogenum* stoll employing agro-based wastes through solid state fermentation: influence of carbon and nitrogen. IOSR Journal of Pharmacy. 2012;2(1):109-112.
41. Steiner J, Socha C, Eyzaguirre J. Culture conditions for enhanced cellulase production by a native strain of *Penicillium purpurogenum.* World Journal of Microbiology and Biotechnology. 1994; 10(3):280-284.
42. Dhake AB, Patil MB. Production of ß-Glucosidase by *Penicillium purpurogenum.* Braz. J. Microbiol. 2005;10(2):170-176. Available:http://dx DOI.org/10.1590/S1517-83822005000200013.
43. Geweely NS. Investigation of the optimum condition and antimicrobial activities of pigments from four potent pigment-producing fungal species. Journal of Life Sciences. 2011;5:697-711.

© 2015 Nwadiaro et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1087&id=8&aid=8817