

Optimization of Physico-Chemical Parameters for Hyper Keratinase Production from a Newly Isolated *Aspergillus* sp. DHE7 using Chicken Feather as Substrate- Management of Biowaste

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ARTICLE INFO

Article history:

Received on: 11/04/2017

Accepted on: 10/07/2017

Available online: 30/09/2017

Key words:

Keratinase; *Aspergillus* sp. DHE 7; optimization; keratinous wastes; biowaste management.

ABSTRACT

Keratinases have recently gained biotechnological impetus due to their ability in degrading insoluble keratinous solid wastes into simplified forms, which used in the preparation of nitrogenous fertilizers, biodegradable films, glues and foils. Keratinases are widespread in nature and mainly produced by diverse microorganisms including bacteria, fungi and actinomycetes. Therefore, the aim of the present work was to isolate and identify a new potent fungal strain possessing extracellular keratinase production capacity using keratinous solid wastes as the sole carbon and nitrogen sources. Fifteen keratinolytic fungi, isolated from a poultry farm soil using white chicken feathers as substrate, were screened qualitatively and quantitatively for their ability to produce extracellular keratinase. Medium optimization for enzyme production was also studied under submerged fermentation conditions. The results revealed that a new fungus identified as *Aspergillus* sp. DHE7 was reported as the most potent fungal strain for extracellular keratinase production under submerged shaking conditions (120 rpm). The highest yield of enzyme of 199 ± 4.2 U/mL was observed after four days of incubation at pH 6.0 and temperature 30 °C using 2 % of chicken feathers as substrate. Medium supplementation with 0.5 % sucrose as an additional source of carbon increased keratinase yield to 226 ± 5.4 U/mL. While using additional nitrogen sources could not promote a favorable effect on keratinase productivity as compared with the basal medium. Different keratinous wastes were used as substrate in the basal medium and better keratinase production was obtained with goat hair medium (452 ± 12.3 U/mL) followed by turkey feather (435 ± 9.2 U/mL) and sheep wool (322 ± 13.4 U/mL) media. The present data suggest *Aspergillus* sp. DHE7 as a potential keratinolytic strain for the bioconversion of keratinous wastes, through a non-polluting process.

INTRODUCTION

Keratins, the insoluble structural proteins of wool and feathers, are one of the most abundant highly rigid biopolymer in the world. They are known for their difficult degradation and high stability since the polypeptides are strongly stabilized and

densely packed by hydrogen bonds and hydrophobic interactions (Brandelli, 2008). Moreover, cross-linkages between the protein chains by disulfide bonds provide high stability and resistance to degradation by the common proteases such as pepsin, trypsin and papain (Brandelli *et al.*, 2010). Keratins are classified into α - and β -types; α - type is predominantly found in wool, hair, horns, claws of mammals and nails, while the harder β - type is commonly found in beaks, claws and feathers of birds (Matikevičiene *et al.*, 2011). Millions of tons of feathers are annually discarded to the environment as a waste byproduct at poultry processing plants, causing serious problems as a pollutant and in the outbreaks of H5N1 virus.

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Hence, it's important to develop methods for remediation of keratin with easy processing set-up and lower cost. Feathers have been treated chemically under steam pressure to reduce the rigidity and increase the digestibility; however, this method has disadvantages because it consumes large amounts of energy and also some heat-sensitive amino acids, i.e. tryptophane and lysine are destroyed during treatment (Kumar *et al.*, 2010). Therefore, enzymatic biodegradation is emerged as an alternative cheap treatment, which improves the nutritional value of feathers and also offers mild conditions for valuable products production. Microbial keratinases, a group of proteolytic enzymes, may meet this preference because they are capable of decomposing complex keratin-rich waste materials to simplified forms and keratinophilic microorganisms including bacteria, fungi and actinomycetes naturally occur on keratinous wastes (Ali *et al.*, 2011; Selvam and Vishnupriya, 2012).

Keratinases from microorganisms are also used in the preparation of protein supplements, animal nutrients, cosmetics, leather manufacture, textile processing, detergent formulation and nitrogenous fertilizer for plants (Goushterova *et al.*, 2005). In addition, keratinases have a prospective application in novel fields such as prion degradation for the treatment of dreaded mad cow disease, production of feather meal and biodegradable plastic manufacture (Gupta and Ramnani, 2006). Therefore, because of the growing interest of microbial keratinases in different industrial sectors, it is essential to search for new keratinolytic microbes that produce novel keratinases with prospective industrial applications. However, the production of keratinase by microorganisms and biodegradation of keratin, vary considerably according to species, culture conditions and keratin substrates as reported by Cai and Zheng (2009).

Thus, the aim of this study was to isolate new potent fungal strains possessing extracellular keratinase production capacity under submerged fermentation conditions using keratinous solid waste as sole carbon and nitrogen sources. Medium optimization for enzyme production and its application in keratin-rich wastes degradation were also investigated.

MATERIAL AND METHODS

Chemicals

Potato dextrose agar (PDA) was obtained from Laboratorios Conda S.A., Spain. Dimethyl sulfoxide (DMSO) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroform and methanol were purchased from Fisher Scientific, UK. Tween- 80 was purchased from Acros Chemical Co., Geel, Belgium.

Substrate

Chicken feathers, kindly obtained from Alwatania Poultry Co., Egypt, were thoroughly washed with tap water, saline solution, rinsed with distilled water and dried under sunlight. The dried feathers were soaked in chloroform: methanol (1:1, v/v), extensively washed with tap water and finally with

distilled water. Feathers were dried in hot air-circulation oven at 60 °C to constant weight, cut into uniform size (1.0 cm length) and stored at 5 °C until used.

Soil sample

Fifteen keratinophilic fungal strains tested in this study were locally isolated from different soil samples collected from poultry farms (El Fayoum, Egypt) by using a sterile scalpel, at 5 cm depth and transferred to a polythene bag. One gram of each soil was transferred in a flask containing 99 mL of sterile distilled water then shaken (Innova® 4230 shaker incubator; New Brunswick Scientific) at 100 rpm for 15 min and the dilution procedure was performed to give up to 10⁻⁸ dilutions (Choi *et al.*, 1999).

Isolation of keratinolytic fungi using agar plate method

With the help of sterile micropipette, 0.1 mL of each diluted sample was transferred onto agar plates medium (contains (g/L): white chicken feather powder; 10, 10 MgSO₄·H₂O; 0.5, KH₂PO₄; 0.1, FeSO₄·7H₂O; 0.01; ZnSO₄·7H₂O; 0.005; streptomycin, 0.005 and agar, 15.0 at pH 6.0) and evenly spread with a sterilized L-rod (Wawrzkiwicz *et al.*, 1991). Keratinolytic activities of the pure isolates were determined as clear zones around the colonies after five days of incubation at 30 °C. The diameter of each clear zone was measured in mm to qualify the activity. Single colonies were picked up and inoculated onto PDA media, incubated for seven days at 28 °C and finally stored at 4 °C.

Detection of aflatoxins

Determination of aflatoxins of the selected fungus was conducted according to AOAC (method 971.24, 2000) through high- performance liquid chromatography technique (1100 HPLC; Agilent Technology, Santa Clara, CA, USA). Millennium Chromatography Manager Software was used to record the observed data.

Preparation of keratin solution

Ten gm of chicken feathers in 500 mL of dimethyl sulfoxide (DMSO) were heated at 100 °C for two h in a reflux condenser. The soluble keratin was precipitated by the addition of chilled acetone (-20 °C) and then kept in refrigerator for two h, followed by cooling centrifugation (refrigerated Sigma 3-18KS centrifuge, Sigma Laborentnugen GmbH, Osterode am Hart, Germany) at 8000×g for ten min.

The precipitate obtained was washed with distilled water and finally dried in a vacuum dryer at 40 °C (Kaul and Sumbali, 1997).

DNA extraction, PCR amplification and phylogenetic tree analysis

The selected fungus was grown in PDA medium for seven days at 28 °C and directly used for 18S rRNA gene amplification by polymerase chain reaction (PCR) technique using

the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Altschul *et al.*, 1997). PCR was performed in 25 μ L as a final volume, which composed of 12.5 μ L of Taq PCR Master Mix (Promega, Germany), containing 0.5 U/ μ L of Taq DNA polymerase, 500 μ M of each dNTP, 20 mM of Tris-HCl (pH 8.3), 100 mM of KCl, 3 mM of each MgCl₂ and Bromophenol Blue, 1 μ L of fungal culture as a template DNA, 1 μ L of each primer (10 μ M) and 9.5 μ L of double distilled H₂O.

The reaction was as follows: denaturation at 95 °C for 1 min followed by annealing at 55 °C for 30 s and extension for two min at 72 °C; and final extension for ten min at 72 °C. The products of PCR were detected by using electrophoresis technique (MSMINIDUO, Clever scientific Ltd., Rugby, Warwickshire, UK) in 1 % agarose gel stained with Tris/Borate/EDTA (TBE) buffer containing ethidium bromide of 1 μ g/mL and finally visualized under UV light, to confirm its purity and size. Core Bio Gel Extraction Kit (RKT13: Chromous Biotech, Karnataka, India) was used to extract DNA fragments from the gel. The fungal gene sequences were compared to other nucleotide sequences of some fungal strains in GenBank database (National Centre for Biotechnology Information) through Basic Local Alignment Search Tool (BLAST) (Benson *et al.*, 2008) and MEGA 4.0 software was used to construct the phylogenetic tree.

Inoculum preparation

The fungal strains were cultivated on PDA slants at 28 °C for seven days. Conidia of each slant were scraped and 5.0 mL of sterile distilled water contains 0.1 % Tween- 80 were added and the final concentration of spore suspension was 1.5×10^6 spore/mL.

Cultivation medium

Feather meal basal medium (FMBM) used in the present study had the following composition (g/L): KH₂PO₄, 1.0; MgSO₄·7H₂O, 0.5; KCl, 0.5 and adjusted to pH 6.0. Aliquots of 50 mL were transferred in 500 mL Erlenmeyer flasks containing 1 gm of chicken feathers, then autoclaved for 15 min at 121 °C.

After cooling, the flasks were inoculated by five mL of spore suspension (1.5×10^6 spore/mL) and incubated under shaking conditions (Innova[®] 4230; New Brunswick TM Scientific, Scituate, MA, USA) at 120 rpm for four days at 28 °C. After the fermentation period, the final medium pH was measured and the hydrolysates were centrifuged (refrigerated Sigma 3-18KS centrifuge, Sigma Laborentnfugen GmbH, Osterode am Hart, Germany) at 5000 \times g for 15 min and filtered (Whatman filter paper no. 1, Inc., Maidstone, UK). The clear supernatants obtained were used for keratinase assay.

Optimization of culture conditions

Medium optimization for extracellular keratinase production by the selected fungal strain was studied by varying one factor at a time, while keeping others as constant.

Fundamental parameters studied include (1) substrate concentration; 5, 10, 20, 30, 40 and 50 g/L of chicken feather, (2) initial pH value of the medium (3.0 - 9.0), (3) time course of keratinase production (during the fermentation, the flasks were taken at regular intervals of 24 h up to 10 days), (4) incubation temperature (25 to 45 °C), (5) supplementation of FMBM medium with additional carbon (glucose, sucrose, fructose, raffinose, arabinos etc at 1 % (w/v)) and nitrogen sources (peptone, malt extract, casein etc at 0.5 % (w/v)). Cultivation was performed under submerged shaking conditions at 120 rpm.

Effect of various types of keratin-rich waste on keratinase production

Different sterile keratinous wastes (duck feather, goose feather, turkey feather and sheep wool) were fragmented into uniform size with about 1 cm long and added individually in the production medium instead of chicken feather. All other optimum culture conditions were performed. At the end of fermentation period, the cultures filtrates were centrifuged at 5000 \times g, filtered through Whatmann filter paper no. 1 and the clear supernatants obtained were used to determine keratinase activity.

Determination of keratinase activity

The enzyme activity was determined according to the method described by Gradisar *et al.* (2005) but with slight modification. Briefly, 1.0 mL of properly diluted crude enzyme extracts in 0.1 M Tris-acetate buffer (pH 8.0) was transferred in a test tube containing 1 mL of soluble keratin solution and incubated for 30 min at 37 °C. The reaction was stopped by the addition of 2.0 mL of trichloroacetic acid (TCA) (15 %), centrifuged at 5000 \times g for 10 min and finally the absorbance of the clear supernatant was measured at 280 nm against a control, i.e. the enzyme solution with 2.0 mL of TCA in absence of the substrate. One unit of keratinolytic activity (U/mL) was defined as an increase of absorbance at A₂₈₀ with that of the control for 0.01 per min under the standard conditions and the following equation was used:

$$U = 4 \times n \times A_{280} / (0.01 \times T)$$

Where 4 is the final reaction volume (mL), *n* is the dilution rate and T is the incubation time (min).

Protein assay

The protein content was determined as described by Bradford (1976) using BSA as a standard.

Statistical analysis

Data reported represent the mean value of three independent trials with standard deviation. The software used to calculate the data was EXCEL 2010 (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

Screening of different fungal strains for the production of extracellular keratinase

The isolation and identification of new keratinolytic fungal strains might help in finding their role in the degradation of insoluble keratin-rich wastes and development of new biotechnological methods with valuable products (Gioppo *et al.*, 2009). In the present research, fifteen fungal strains locally isolated from poultry farm soil samples were qualitatively evaluated for their abilities to grow on chicken feathers as a sole source of carbon and nitrogen. The data reported revealed that most of the isolated fungal strains could grow under these conditions, however, isolate DHE7 was found as the highest keratinase-producing strain since it degraded chicken feathers completely (Fig. 1) and also exhibited the highest clear zone diameter (47 mm) (Table 1).



Fig. 1. Efficiency of *Aspergillus* sp. DHE7 in degrading white chicken feathers after 4 days of incubation at 30 °C under submerged fermentation (SmF) conditions

Table 1 Screening of locally isolated filamentous fungi qualitatively and quantitatively for extracellular keratinase production capacity.

Fungus	Qualitative analysis clear zone diameter (mm)	Protein content (mg/mL)	Quantitative analysis Keratinase activity (U/mL)
Isolate DHE6	14	2.3	62±3.9
Isolate DHE7	47	1.9	168±4.7
Isolate DHE8	-	4.3	15±2.8
Isolate DHE9	33	2.7	116±3.1
Isolate DHE10	-	1.2	59±1.4
Isolate DHE11	12	2.1	37±2.5
Isolate DHE12	28	3.2	127±2.7
Isolate DHE13	35	2.5	123±3.6
Isolate DHE14	-	1.1	21±0.5
Isolate DHE15	32	1.7	154±2.8

Data are the mean value of independent triplicate measurements ±SD.

- = no clear zone.

All fungal strains were quantitatively screened for keratinolytic activity in submerged fermentation conditions using chicken feathers as substrate in the basal medium. Results in Table 1, shown that the highest keratinase production was observed with

isolate DHE7 (168±4.7 U/mL) followed by DHE15 (154±2.8 U/mL), DHE12 (127±2.7 U/mL) and DHE13 (123±3.6 U/mL), which are much higher than data reported by Cai and Zheng (2009) and Mazotto *et al.* (2013) for extracellular keratinase production by *Bacillus subtilis* KD-N2 (75 U/mL) and *A. niger* 9D40 (21.3 U/mL), respectively. Therefore, this promising result justified the selection of strain DHE 7 for the following experimental studies.

Aflatoxins detection

Toxicity test was studied in the present work and the observed results revealed that strain DHE 7 was a non-mycotoxin-producing fungus, which confirm its selection.

Identification based on 18S rRNA gene

A single band of amplified DNA product of 1.3 kbp was reported and the close relatives, based on 18S rRNA gene sequence similarities, were *Aspergillus parasiticus* (D63699.1) (94 %), *A. flavus* (GU953210.1) (94 %) and *A. sojae* (KF175513.1) (94 %). Therefore, this result suggests strain DHE7 as a new *Aspergillus* sp. within family of *Aspergillaceae*, hence the 18S rRNA genes sequences were deposited under the accession number KX950801 to GenBank (Fig. 2) (<http://www.ncbi.nlm.nih.gov>) (accessed November 2016). In this concern, Marcondes *et al.* (2008) investigated that genera *Acremonium*, *Alternaria*, *Beauveria*, *Aspergillus*, *Curvularia*, *Penicillium* and *Paecilomyces* were the most potent filamentous fungi for keratinase production when grown on poultry feather as a substrate.

Optimization of various physiological parameters

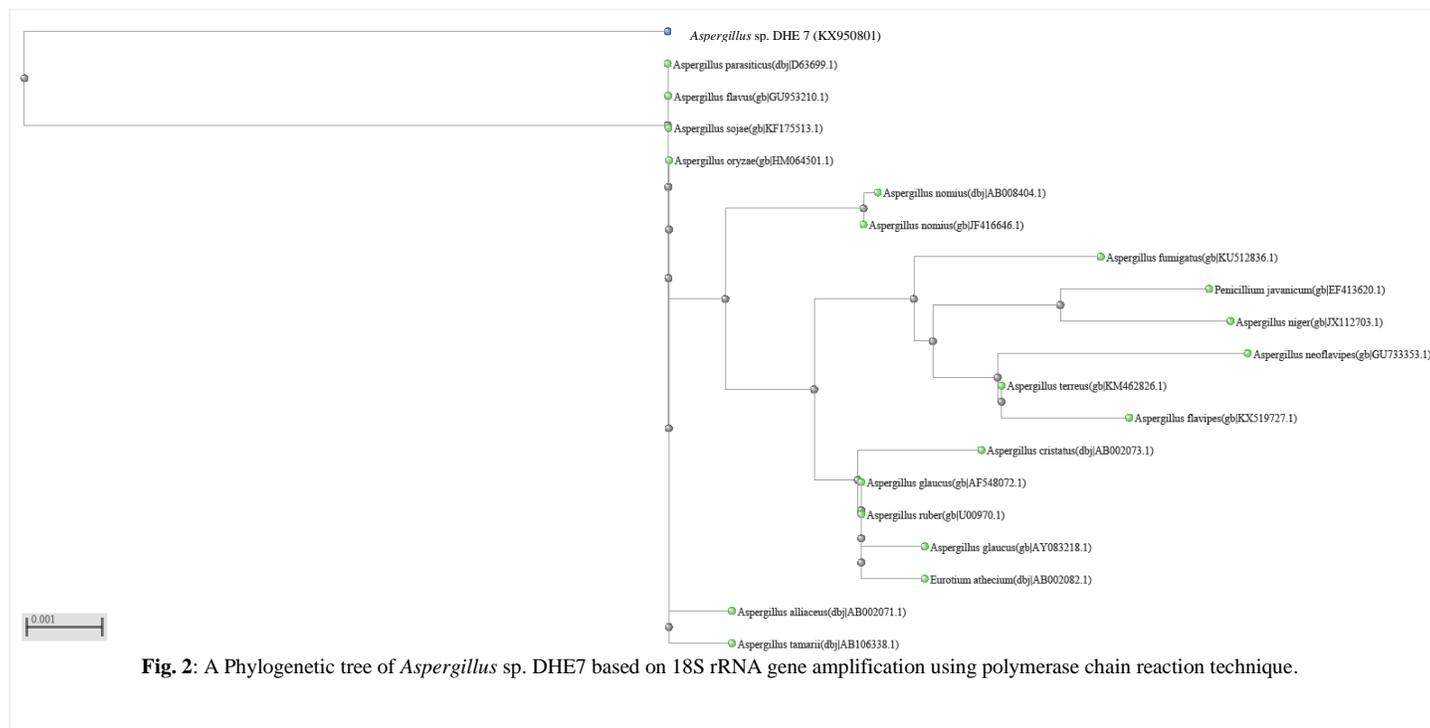
Substrate Concentration

Keratinolytic microorganisms are known for their capabilities of using keratin-rich substrates as carbon and nitrogen sources, whereas, the carbon-free medium forces the fungus to utilize keratin and subsequently the percentage of solubilization increased (Goushterova *et al.*, 2005). In the present experiment, chicken feather concentrations of 5, 10, 20, 30, 40 and 50 g/L were evaluated for maximum keratinase production by *Aspergillus* DHE 7 and the reported data revealed that the production of keratinase by the fungus increased with the increase in feather concentration up to 20 g/L with maximum production of 164.3±3.6 U/mL (Table 2).

Table 2: Effect of different concentrations of feather on keratinase production by *Aspergillus* sp. DHE7 in submerged fermentation (SmF).

Feather concentration (%)	Protein content (mg/mL)	Keratinase activity (U/mL)
0.5	0.8	89±2.1
1.0	1.3	127±3.3
2.0	1.8	164±3.6
3.0	2.9	128±3.2
4.0	3.2	103±2.7
5.0	3.9	98±1.6

Data are the mean value of independent triplicate measurements ±SD.



However, at higher concentration a gradual decrease in enzyme production was investigated. Similar results were reported by Suntornsuk and Suntornsuk (2003) for keratinase production by *Bacillus* sp. FK 46. They reported that higher feathers concentrations (30-50 g/L) resulted in repression of keratinase production. In contrast, Cai *et al.* (2008) reported that 10 g/L of feather was the optimal concentration for the production of keratinase by *Bacillus subtilis*.

Effect of initial pH value

The reported data in Fig. 3 represent the influence of various pH values on keratinase production by *Aspergillus* sp. DHE7 after 4 days of incubation at 30 °C. Enzyme production was considerably decreased at lower pH values and enhanced at higher ranges. However, the optimal pH for enzyme production (163 ± 2.7) was observed at pH 6.0. This result is congruent with several studies reported on keratinolytic microorganisms and their biotechnological potential. In most keratinophilic microorganisms, neutral to alkaline pH values (6.0 to 9.0) have been reported as the most suitable for keratinase production and feather degradation, which might be attributed to the modification in cystine residues to lathionine at alkaline pH, which makes it accessible for keratinase action (Gupta and Ramnani, 2006; El-Gendy, 2009; Jain and Sharma, 2012). On the other hand, keratinase production by few microorganisms were found to be optimal at higher pH values (Gessesse *et al.*, 2003).

Time course

Incubation period is considered as an essential parameter for optimal keratinase production by microorganisms when grown under submerged fermentation conditions. In the present work, the

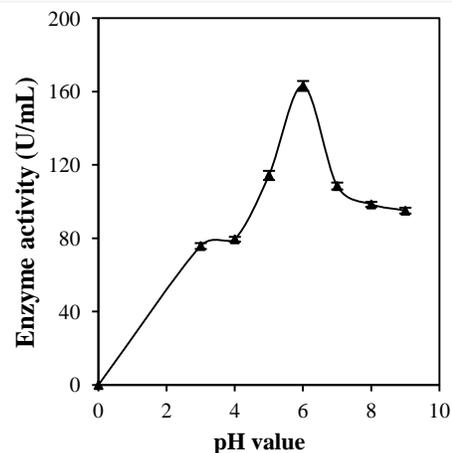


Fig. 3: Influence of pH on keratinase production by *Aspergillus* sp. DHE7.

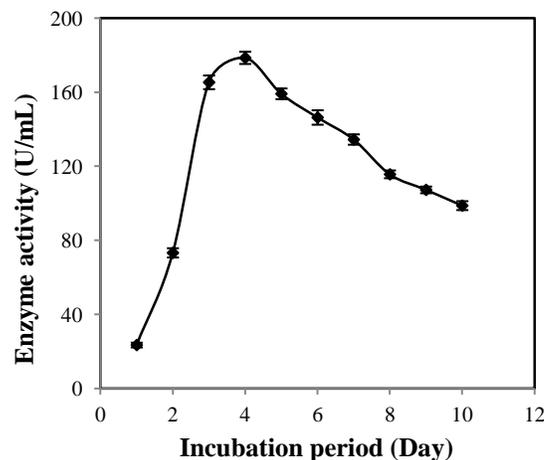


Fig. 4: Influence of incubation period on the production of keratinase by *Aspergillus* sp. DHE7.

aerobic growth of *Aspergillus* sp. DHE7 on chicken feathers as a sole source of nutrition resulted in complete degradation of feathers after 72 h of fermentation. In addition, the analysis of culture supernatant showed that the enzyme activity increased from an initial of 23±1.3 U/mL after 24 h of incubation giving its optimal activity of 179±3.3 U/mL on the fourth day of fermentation (Fig. 4). However, the extension of incubation period was accompanied with a reduction of enzyme production, which could be either due to the inactivation of the enzyme in the presence of some kinds of proteolytic activities or the growth of the fungus reached a stage after which it could not balance its steady growth with the nutrient resources available. Keratinolytic enzymes are mostly synthesized by the microorganism during its late exponential phase or the stationary phase growth (Thys *et al.*, 2004). In accordance with our result, Noronha *et al.* (2002) observed optimum keratinase from *A. fumigatus* after 72 h of incubation. Similarly, Kanchana (2012) reported optimal keratinase production from *Bacillus* sp. at 37 °C after 72 h of incubation when grown under submerged culture fermentation. On the other hand, optimum keratinase production from *Penicillium* sp. was reported on the fifth day (El-Gendy, 2009). Matikevičiene *et al.* (2009) investigated maximum keratinase production by *Bacillus* sp. after 24 h of incubation.

Keratinase production in relation to incubation temperature

Most microorganisms (bacteria, actinomycetes and fungi) produce keratinase at temperature ranges from 28 to 50 °C (Nam *et al.* 2002). The reported results in Table 3 clearly indicated that maximum keratinolytic activity was obtained between 25-35 °C, with optimal enzyme production at 30 °C (176±3.4 U/mL). This result is similar to that reported for keratinase production by *Penicillium* sp. (El-Gendy, 2009). On the contrary, Bockle and Muller (1997) and Anbu *et al.* (2008) reported optimum keratinase production by *Streptomyces pactum* and *Trichophyton* sp., respectively, at higher temperature ranges near 40 °C.

Table 3 Effect of incubation temperature on the production of keratinase from *Aspergillus* DHE7 grown under submerged fermentation (SmF) conditions.

Temperature (°C)	Protein content (mg/mL)	Keratinase activity (U/mL)
25	1.4	132±1.3
30	1.7	176±3.4
35	1.8	155±4.2
40	0.8	99±2.7
45	0.6	73±3.1

Data are the mean value of independent triplicate measurements ±SD.

Influence of additional carbon source on keratinase production

In the search to improve the enzyme production by *Aspergillus* sp. DHE7, different carbon sources were added separately in the basal medium, while the medium free from the additional carbon source was served as the control. As depicted in Fig. 5, among all the carbon sources tested, sucrose proved to be the best for keratinase production yielding 226±4.6 U/mL followed by arabinose (198±2.5 U/mL) and glucose (195±3.1

U/mL), while maltose, raffinose and galactose negatively modulated keratinase production. This result is congruent with that reported by Cai and Zheng (2009) for the production of keratinase by *Bacillus subtilis* KD-N2. Similarly, Malviya *et al.* (1992) and El-Naghy *et al.* (1998) reported maximum keratinase production from *Chrysosporium queenslandicum* and *C. georgiae*, respectively, in the presence of keratin, which was further stimulated by the addition of glucose. On the contrary, production of keratinase by *Streptomyces* sp. MS-2 was highly inhibited in the presence of starch and glucose as reported by Mabrouk (2008). Thys *et al.* (2004) suggested that the suppression of glucose to keratinase synthesis is due to the catabolite repression.

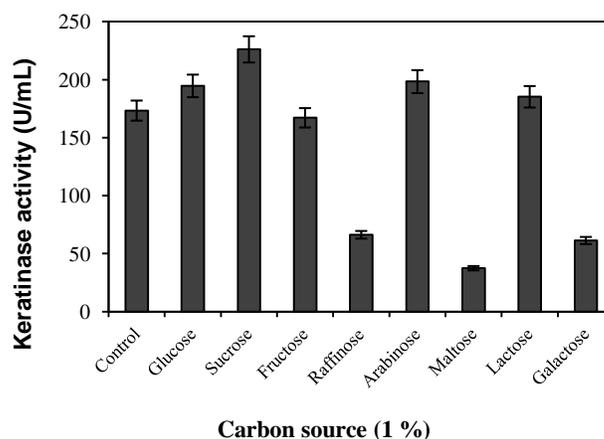


Fig. 5: Effect of carbon source on the production of keratinase by *Aspergillus* sp. DHE7

Our results also showed that culture grown in 0.5 % of sucrose exhibited maximum keratinase production by *Aspergillus* sp. DHE7 grown under submerged fermentation (Fig. 6), while at higher concentrations, sucrose acts as a repressor for enzyme production.

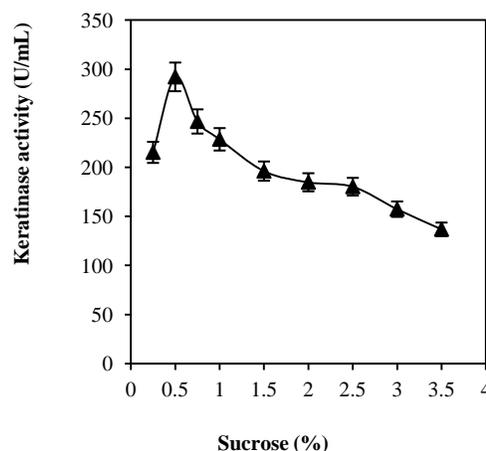


Fig. 6: Effect of different concentrations of sucrose on keratinase production by *Aspergillus* sp. DHE7.

Role of nitrogen supplementation on the production of keratinase

The reported results in Fig. 7 showed that none of the tested nitrogen sources could exert a favorable effect on enzyme

production by *Aspergillus* sp. DHE7 as compared with the basal medium (without the tested nitrogen source). Similarly, Santos *et al.* (1996) reported that nitrates had negative effects on keratinase production by *Aspergillus fumigatus*. El-Naghy *et al.* (1998) and Son *et al.* (2008) reported a partial or complete repressive effect on keratinase production by *Chrysosporium georgiae* and *Bacillus pumilis*, respectively, in cultures supplemented with different nitrogen molecules, which might be attributed to the catabolite repression exerted by these additional nitrogen sources (Ulfig *et al.*, 2003; Gioppo *et al.*, 2009). On the other hand, Veselal and Friedrich (2009) reported that the biodegradation of different keratinous wastes by *Paecilomyces marquandii* was enhanced in the presence of additional nitrogen sources.

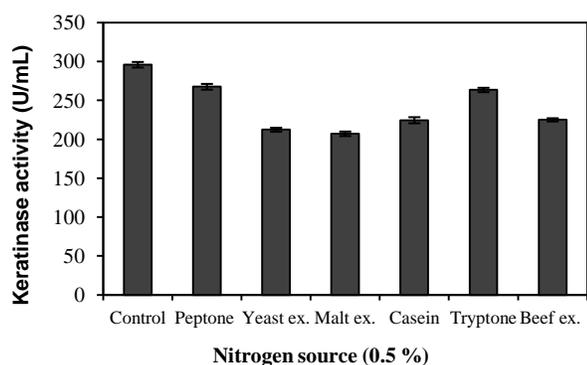


Fig. 7: Effect of additional nitrogen source on keratinase production by *Aspergillus* sp. DHE7.

Biodegradation of keratinous solid wastes by *Aspergillus* sp. DHE7

Different keratinous wastes were used as substrate in the growth medium of the tested fungal strain. A comparison of the enzyme production by the selected fungal strain when grown on different keratin-rich substrates is presented in Table 4. *Aspergillus* sp. DHE7 possessed better keratinase production in the presence of goat hair (452±12.3 U/mL), turkey feather (435±9.2 U/mL) and sheep wool (322±13.4 U/mL). The degradation of such keratinous wastes by the filamentous fungus might be resulted from the combination effect of extracellular keratinase, sulphitolysis and proteolysis (Szabo *et al.*, 2000; Gupta and Ramnani, 2006). In addition, Gioppo *et al.* (2009) reported that the initial attack of keratinous substrate by keratinases followed by disulfide reductases may permit other proteases to act, which results in an extensive hydrolysis of keratinous wastes.

Table 4: Biodegradation of various types of keratinous waste by *Aspergillus* sp. DHE7.

Keratinous waste (2%)	Final pH	Protein content (mg/mL)	Keratinase activity (U/mL)
Chicken feather	9.3	1.7	298±4.1
Duck feather	8.1	1.5	158±2.8
Goose feather	7.9	2.4	301±3.4
Turkey feather	7.7	2.7	435±9.2
Pigeon feather	9.2	2.1	276±2.7
Goat hair	6.6	3.1	452±12.3
Sheep wool	9.2	3.4	322±13.4

Data are the mean value of independent triplicate measurements ±SD.

CONCLUSIONS

Keratinolytic filamentous fungi are of great ecological importance not only in pathogenesis but also in the degradation of keratin residues. In the search for new source for keratinases, different fungi were isolated from poultry farm soil and a new isolate identified as *Aspergillus* sp. DHE7, was reported as the potent fungus for keratinase production under SmF. Maximum enzyme production from the fungal strain was reported when grown in a medium at an initial pH value of 6.0, at temperature 30 °C, for four days under shaking at 120 rpm using chicken feathers as substrate (20 g/L). Different carbon sources could enhance keratinase production by *Aspergillus* sp. DHE7, with sucrose being the optimal. On the other hand, maltose, raffinose and galactose negatively modulated keratinase production. It is worth mentioning that none of the tested nitrogen sources could exert a favorable effect on the production of keratinase. Different keratinous wastes were used as substrate in the growth medium and better keratinase production from *Aspergillus* sp. DHE7 was reported with goat hair medium followed by turkey feather and sheep wool containing media. The present findings revealed *Aspergillus* sp. DHE7 to be a potential organism for keratinase production and in the management of keratinous solid wastes.

ACKNOWLEDGEMENTS

Financial support and sponsorship: Authors are thankful for National Research Centre (NRC), Egypt, for the financial support (Project number: P101117).

Conflict of Interests: There are no conflicts of interest.

REFERENCES

- Ali TH, Ali NH, Mohamed LA. Production, purification and some properties of extracellular keratinase from feathers-degradation by *Aspergillus oryzae* NRRL- 447. J Appl Sci Environ Sanit Sby, 2011; 6:123-136.
- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997; 25:3389-402.
- Anbu P, Hilda A, Sur HW, Hur BK, Jayanthi S. Extracellular keratinase from *Trichophyton* sp. HA-2 isolated from feather dumping soil. Int Biodeterior Biodegrad, 2008; 62:287-292.
- AOAC, Official Method 971.24: Aflatoxins in coconut, copra, and copra meal. AOAC International; Rockville, MD, USA: 2000.
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Wheeler DL. GenBank. Nucleic Acids Res. 2008; 36:D25-30.
- Bockle B, Muller R. Reduction of disulfide bonds by *Streptomyces pactum* during growth on chicken feathers. Appl Environ Microbiol, 1997; 63:790-792.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976; 72:248-254.
- Brandelli A, Daroit DJ, Riffel A. Biochemical features of microbial keratinases and their production and applications. Appl Microbiol Biotechnol, 2010; 85:1735-1750.
- Brandelli A. Bacterial keratinases: useful enzymes for bioprocessing agro-industrial wastes and beyond. Food Bioprocess Technol, 2008; 1:105-116.

Cai C, Zheng X. Medium optimization for keratinase production in hair substrate by a new *Bacillus subtilis* KD-N2 using response surface methodology. *J Ind Microbiol Biotechnol*, 2009; 36:875-883.

Cai CG, Lou BG, Zheng XD. Keratinase production and keratin degradation by a mutant strain of *Bacillus subtilis*. *J Zhejiang Univ Sci, B*, 2008; 9:60-67.

Choi YW, Hyde KD, Ho WH. Single spore isolation of fungi. *Fungal Diversity*, 1999; 3:29-38.

El-Gendy MMA. Keratinase production by endophytic *Penicillium* sp. Morsy1 under solid-State fermentation using rice straw. *Appl Biochem Biotechnol*, 2009; 78:54-63.

El-Naghy MA, El-Ktatny MS, Fadel-Allah EM, Nazeer WW. Degradation of chicken feathers by *Chrysosporium georgiae*. *Mycopathologia*, 1998; 143:77-84.

Gessesse A, Hatti-Kaul R, Gashe BA, Mattiasson B. Novel alkaline proteases from alkaliphilic bacteria grown on chicken feather. *Enzyme Microb Technol*, 2003; 32:519-524.

Gioppo NMR, Gasparin FGM, Costa AM, Alexandrino AM, de Souza CGM, Peralta RM. Influence of the carbon and nitrogen sources on keratinase production by *Myrothecium verrucaria* in submerged and solid state cultures. *J Ind Microbiol Biotechnol*. 2009; 36:705-711.

Goushterova A, Braikova D, Goshev I, Christov P, Tishinov K. Degradation of keratin and collagen containing wastes by newly isolated thermoactinomycetes or by alkaline hydrolysis. *Lett Applied Microbiol*, 2005; 40:335-340.

Gradisar H, Friedrich J, Križaj I, Jerala R. Similarities and specificities of fungal keratinolytic proteases: comparison of keratinase of *Paecilomyces marquandii* and *Doratomyces microsporus* to some known proteases. *Appl Environ Microbiol*, 2005; 71:3420-3426.

Gupta R, Ramnani P. Microbial keratinases and their prospective applications: An overview. *Appl Microbiol Biotechnol*, 2006; 70:21-33.

Jain N, Sharma M. Biodiversity of keratinophilic fungal flora in university campus, Jaipur, India. *Iranian J Public Health*, 2012; 41:27-33.

Kanchana R. Farm waste recycling through microbial keratinases. *J Appl Sci Env Sanitation*, 2012; 7:103-108.

Kaul S, Sumbali G. Keratinolysis by poultry farm soil fungi. *Mycopathologia*, 1997; 139:137-40.

Kumar RS, Balaji MS, Karthikeyan M. Purification and characterization of an extracellular keratinase from a horn meal-degrading *Bacillus subtilis* MTCC. *World J Microbiol Biotechnol*, 2010; 24:2741-2745.

Mabrouk MEM. Feather degradation by a new keratinolytic *Streptomyces* sp. MS-2. *World J Microbiol. Biotechnol*, 2008; 24:2331-2338.

Malviya HK, Rajak RC, Hasija SK. Synthesis and regulation of extracellular keratinase in three fungi isolated from the grounds of a gelatin factory, Jabalpur, India. *Mycopathologia*, 1992; 120:1-4.

Marcondes NR, Taira CL, Vandresen DC, Svidzinski TIE, Kadowaki MK, Peralta RM. New feather-degrading filamentous fungi. *Microb Ecol*, 2008; 56:13-19.

Matikevičiene V, Masiliuniene D, Grigiskis S. Degradation of keratin containing wastes by bacteria with keratinolytic activity, in Proceedings of the 7th International Scientific and Practical Conference, 2009; 1:284-289.

Matikevičiene S, Grigiskis D, Levišauskas K, Sirvydytė O, Dižavienė D, Masiliūnien U, Ancenko O. Optimization of keratinase production by *Actinomyces fradiae* 119 and its application in degradation of Keratin containing wastes. *Env Technol Resource*, 2011; 1:294-300.

Mazotto AM, Couri S, Damaso M, Vermelho AB. Degradation of feather waste by *Aspergillus niger* keratinases: Comparison of submerged and solid-state fermentation. *Int Biod Biod*, 2013; 85:189-195.

Nam GW, Lee Dong W, Lee Nam J, Kim B, Young C, Choe EH, et al. Native feather degradation by *Fervidobacterium islandicum* AW-1, a newly isolates keratinase producing thermophilic anaerobe. *Arch Microbiol*, 2002; 178:538-547.

Noronha EF, de Lima BD, de Sa CM, Felix CR. Heterologous production of *Aspergillus fumigatus* keratinase in *Pichia pastoris*. *World J Microbiol Biotechnol*, 2002; 18:563-568.

Santos RMDB, Firmino AA, de Sa' CM, Felix CR. Keratinolytic activity of *Aspergillus fumigatus* Fresenius. *Curr Microbiol*, 1996; 33:364-370.

Selvam K, Vishnupriya B. Biochemical and molecular characterization of microbial keratinase and its remarkable applications. *Int J Pharm Biol Arc*, 2012; 3:267-275.

Son HJ, Park HC, Kim HS, Lee CY. Nutritional regulation of keratinolytic activity in *Bacillus pumilis*. *Biotechnol Lett*, 2008; 30:461-465.

Suntornsuk W, Suntornsuk L. Degradation by *Bacillus* sp. FK46 in submerged cultivation. *Bioresour Technol*, 2003; 86:239-43.

Szabo I, Benedek A, Szabo MI, Barabas G. Feather degradation with a thermotolerant *Streptomyces graminofaciens* strain. *World J Microbiol Biotechnol*, 2000; 16:253-255.

Thys RCS, Lucas FS, Riffel A, Heeb P, Brandelli A. Characterization of a protease of a feather-degrading *Microbacterium* species. *Lett Appl Microbiol*, 2004; 39:181-186.

Ulfig K, Plaza G, Worsztynowicz A, Mańko T, Tien AJ, Brigmon RL. Keratinolytic fungi as indicators of hydrocarbon contamination and bioremediation progress in a petroleum refinery. *Polish J Environmental Studies*, 2003; 12:245-250.

Vesela M, Friedrich J. Amino acid and soluble protein cocktail from waste keratin hydrolysed by a fungal keratinase of *Paecilomyces marquandii*. *Biotechnol Bioprocess Eng*, 2009; 14:84-90.

Wawrzkiwicz K, Wolski T, Lobarzewski J. Screening the keratinolytic activity of dermatophytes *in vitro*. *Mycopathologia*, 1991; 114:1-8.

How to cite this article:

El-Ghonemy DH, Ali TH. Optimization of Physico-Chemical Parameters for Hyper Keratinase Production from a Newly Isolated *Aspergillus* sp. DHE7 using Chicken Feather as Substrate-Management of Biowaste. *J App Pharm Sci*, 2017; 7 (09): 171-178.