J. Agric. & Env. Sci. (Damanhour University) 2022, 21(2): 1-18
Print: ISSN 1687-1464 Online: 2735-5098

FEATHER DEGRADING FUNGI: ISOLATION, IDENTIFICATION AND MEASURING THE PROTEOLYTIC ACTIVITY USING SOLID-STATE FERMENTATION TECHNIQUE

Neama Derhab*, Mohammad M. El-Metwally, Mona E.M. Mabrouk, Youssef M.M. Mohammad
Department of Botany and Microbiology, Faculty of Science, Damanhour University, Damanhour, Egypt
***Corresponding author:** Email: neama844@sci.dmu.edu.eg
***ORCID**: https://orcid.org/0000-0001-8901-4289

ABSTRACT

The rapid growth of the poultry industry has been associated with an increase in the production of keratin-containing residues (feathers are most abundant). Keratinolytic microorganisms have become biotechnologically important since they target the hydrolysis of highly rigid keratin. The main aim of the recent study was to isolate *Aspergilli* that have a keratinolytic activity. The keratinolytic fungi were isolated from three different places containing keratinous wastes. For cultivating these organisms, a solid-state fermentation (SSF) media was used containing feather as the only source of carbon and nitrogen. Then, we used Potato dextrose agar (PDA) media for purification and identification of isolates, by studying their culture and microscopic characteristics. Based on our results, we obtained four keratinolytic fungal species; namely, *Aspergillus terreus, Aspergillus nidulans, Aspergillus niger*, and *Aspergillus fumigatus*. The total protein content in the

fermented media was measured using the method of Lowry. The hydrolysis of feathers by fungi resulted high amount of soluble protein from the insoluble hard keratin presented in chicken feather, which can be used as an organic fertilizer or as an ingredient in animal feed, reducing the environmental impact of feather waste from the chicken industry. This study highlights the feather-degrading fungi that are useful as biotechnological solutions, in the management of keratinous waste by using environmentally friendly methods.

Keywords: Keratinolytic fungi, feather bio-degradation, keratin, keratinase, *Aspergillus* sp.**,** solid-state fermentation

INTRODUCTION

Every year, the meat processing, poultry, leather and wool sectors produce million tons of keratin waste. The global poultry manufacturing sector produces 40×10^6 tons of feather wastes. Chemical hydrolysis, incineration or burial is used in organized landfills to dispose of residual garbage (Tesfaye et al., 2017). However, their widespread usage is limited due to high operating costs, significant energy consumption, and the loss of essential bio-resources (Gupta & Ramnani, 2006). Inappropriate disposal of waste leads to environmental damage and disease transmission. The environmental interesting in using renewable and sustainable raw materials beside the need to reduce dependence on non-renewable petroleum resources, encourage the industry to develop better solutions to deal with waste (Elkhwesky, 2022; Elkhwesky et al., 2022; Elkhwesky & Elkhwesky, 2022; Elkhwesky et al., 2022; García et al., 2022) such as feather.

Keratin biomass is one of the most complicated biological materials; it is responsible for the majority of skin appendages such as nails and hair, tortoiseshells, horns, Claws, beaks, and feathers (Wang

et al., 2016). Despite the fact that keratin-containing by-products are produced in poultry, pig, and cattle husbandry, but chicken feathers are the most abundant type of keratin residue (Fagbemi et al., 2020). Chicken feather considers a highly source of protein called keratin that can be converted into a variety of high value bio-products (Tesfaye et al., 2017).

Keratin has a very complex structure that includes super-coiled polypeptide chains, hydrophobic interactions, cross linking hydrogen bonds, and disulfide bridges formed between cysteine residues within and between keratin polypeptides. These reasons giving keratin mechanical stability and resistance to traditional proteolytic enzymes (pepsin, trypsin, and papain) and also chemical agents (Gupta et al., 2013; Callegaro et al., 2019). Because of their recalcitrant character, these proteins are difficult to digest by traditional proteases; however, nature has provided a brilliant solution to this problem in the form of keratinolytic enzymes. Researchers are interested in using keratin-degrading microorganisms to clean feathers as they can completely hydrolyze keratin under minor conditions and produce better hydrolysate rich in amino acids and peptides (Schor & Krimm, 1961; Brandelli et al., 2015; Qiu et al., 2020).

These keratinolytic enzymes are secreted by different types of microorganisms; come from bacteria such as *Bacillus subtilis* and *B. licheniformis.* Moreover, the actinobacteria *Streptomyces* also secrete keratinases and fungal keratinases are mainly from*Trichophyton* sp. and *Microsporum sp.* (Qiu et al., 2020). The tight attachment of filamentous fungi and penetration of keratin substrates by mycelium is a morphological property of filamentous fungus that enhances keratin degradation. The best habitat for keratinolytic microbial samples including feather dumping sites, poultry manufacturing, and tannery/slaughterhouse discharges (Bhari et al., 2021).

Microbial keratinases have been accepted for numerous potential application as follows; medical, biotechnological and industrial fields, bioremediation process in wastes management, food, animal feed, detergent formulation, cosmetic formulations (hair-removing creams and lotions, etc.), textile, biofertilizers (nitrogen and different minerals rich) production, manufacturing of biodegradable films, fiber modification, and industrial of glues by hydrolyzing the keratin substrates (Verma et al., 2017; Sharma & Devi, 2018; Vidmar & Vodovnik, 2018; Tamreihao et al., 2019). In our study, keratinolytic species were isolated from keratinous waste area, and study the activity of degrading keratin and produce soluble protein.

MATERIALS AND METHOD

Soil samples collection

Various samples were taken from different sites such as leather store, slaughter house and compost; 1 g of each samples were serially diluted separately in 9 ml sterilized distilled water**,** shaken at 150 rpm (revolutions per minute) for 5 min the resulted suspension were used as a source for fungal isolates.

Isolating of keratinolytic fungal strain

Using solid state fermented media with feather powder by products from process of chicken manufacturing as a sole carbon and nitrogen source. The component media in each isolated plate containing; 10 gm chicken feather powder after autoclave sterilization adding 5ml sterilized distilled water using for moistening and 0.5 ml antibacterial solution of Amoxicillin and Flumox (5 mg/ml), and inoculated with 1 ml of (10^{-2}) diluted sample and incubated for a weak at 28°C in a static conditions**.**

Purification of fungal isolates

A mixture of various soil-borne fungi was grown on solid state feather media, and one colony of each fungus transferred to Potato Dextrose Agar (PDA) media plate by a sterilized needle for isolation of pure culture. After ensuring purity, the cultures were sub cultured on PDA agar slants and stored at 4ºC as stock cultures.

Identification of fungal isolates

The isolated fungi were sub cultured on PDA agar and allowed to grow and sporulation for studding their colony and morphological characteristics. Lacto phenol cotton blue stain used as the mounting fluid for microscopic characterization, the slides observed under the microscope then fungi were identified by following the mycological literature with the help of standard fungal identification manuals.

Screening the proteolytic activity of keratinolytic fungi

The keratinolytic isolated fungi were inoculated into solid state fermented media containing feather as the sole source of carbon and nitrogen, as follow: grinded chicken feathers (0.1 g/flask);with 0.5 ml of moistening solution (g/l): K_2HPO_4 6.3; KH_2PO_4 1.8; $MgSO_4$-1.0; $FeSO_4$ 0.1 and $MnSO_4$ 0.1(Mini et al., 2015), dissolved in tab water. The flasks were sterilized by autoclave, then inoculated with 0.5 ml of fresh spore suspension of the isolated fungi, and incubated at 30 ◦C statically incubation for 6 days.

Extraction of culture filtrate

For extracting the culture filtrate, adding 10 ml of sterile distilled water to each flask, the flasks were saved in the shaking incubator at 150 rpm for 30 min at 30° C, then the contents were

separated using centrifugation, and the cell-free supernatants were utilized for measuring protein concentration.

Protein assay

The total protein content in the fermented media which resulted from feather degradations by the growth of keratinolytic fungi was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as a protein standard curve.

RESULTS

Isolating of keratinolytic fungal strain

Keratinolytic fungal isolates were isolated from some keratinous waste area like; compost, leather store and slaughter house. They showed growth and degradation on chicken feather media after a week of incubation (Figure 1).

Figure 1. Source of fungal isolation samples: (A) control plate, (B) compost, (C) leather store, and (D) slaughter house.

Purification of fungal isolates

Four species belonging to the genus *Aspergillus* were isolated from soils of; compost, slaughter house and leather store which inoculated on chicken feather media (Table 1). They are purified and

identified as; *Aspergillus terreus, Aspergillus nidulans, Aspergillus niger* and *Aspergillus fumigatus* by using their morphological and microscopic characters (Figure 2).

Tables 1. Source of keratinolytic fungal isolates

Species of fungi	**Source of isolates**
Aspergillus fumigatus	Natural compost
Aspergillus nidulans	Natural compost
Aspergillus niger	Feather of slaughter house
Aspergillus terreus	Leather store

J. Agric. & Env. Sci. (Damanhour University) 2022, 21(2): 1-18
Print: ISSN 1687-1464 Online: 2735-5098

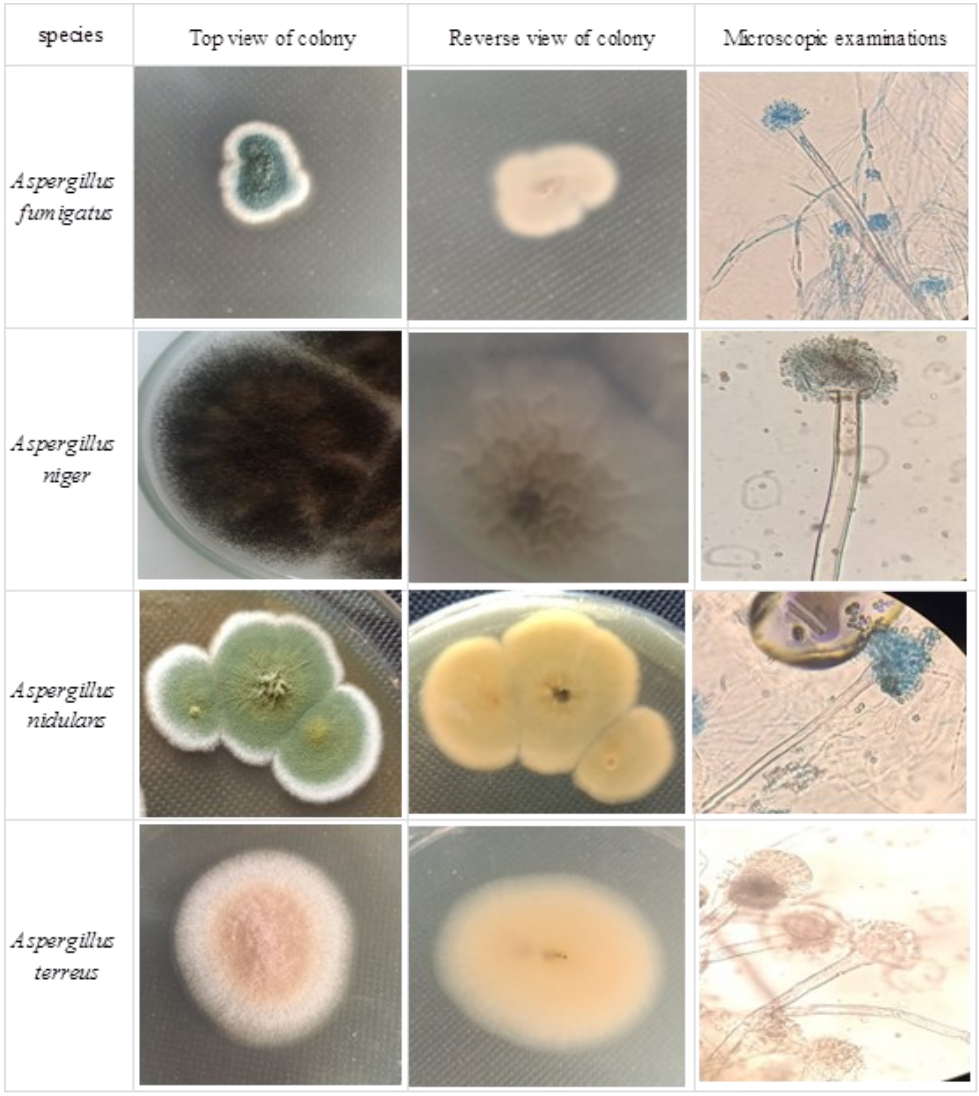

Figure 2. Keratinolytic fungal species were identified as; *Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans* and *Aspergillus terreus.*

Screening for soluble protein production

Total protein concentrations of the four isolates were determined in the solid state fermentation media using feather as the sole source of carbon and nitrogen; all isolates could produce soluble protein from hard insoluble keratin feather (Figure 3). The total amount of protein ranging from 276 to 486 (µg/ml) for the isolates *Aspergillus nidulans* and *Aspergillus niger* respectively.

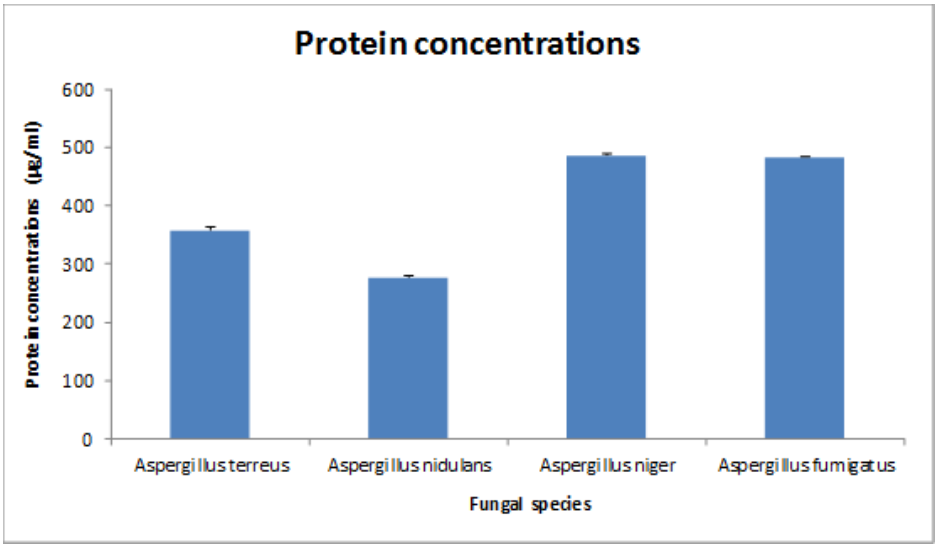

Figure 3. Protein concentrations of fermented media resulted from the activity of keratinolytic fungi (µg/ml)

DISCUSSION

In Egypt as the context our study, the impact of severe pollution of various wastes is a very serious problem; The disposal of huge quantities of chicken feathers and other wastes produced by the poultry industry is increasingly becoming a major problem, and no suitable strategy has been developed to deal with this waste with a dual benefit; the first being the safe disposal of poultry waste and the second being its recycling into valuable by-products (Isaac & Abu-Tahon, 2016). The poultry industry waste is a rich medium that encourages the growth and proliferation of many fungal species (Shaaban et al., 2022). Fungal keratinases satisfy the demands of researchers due to lower cost of production, easy product recovery and purity, higher growth on cheap and readily available substrates, and the release of large amounts of enzymes into the growth medium (Bagewadi et al., 2018).

The present study indicated four fungi viz. *Aspergillus terreus, Aspergillus nidulans, Aspergillus niger* and *Aspergillus fumigatus*, that could grow on chicken feather and degrade it. Similarly, Kim (2003) recorded only five fungi belong to *Aspergillus* genera namely; *A.flavus, A.fumigatus, A.niger, A.nidulans*, and *A.terreus* that could be isolated by grown on chicken feather. However, our study use the condition of solid state fermentation which requires little water consumption and as a result, produces slight waste water and does not require the use of antifoams, which is an advantage of this environmentally friendly method.

Solid-state fermentation (SSF) for keratinase production could be verified by *A. niger*, especially with strain 3T5B8, which showed 7 times more SSF keratinolysis activity than submerged fermentation (SmF) (Mazotto et al., 2013). And recorded by *Aspergillus oryzae*

NRRL 2220 nine times higher neutral peptidase production in SSF compared to liquid culture (Belmessikh et al., 2013). Moreover, when *Trichoderma harzianum* is grown on feather meal as a substrate for solid state fermentation, keratinase synthesis increases seven times compared to submerged cultivation, which is common in industry (Bagewadi et al., 2018). As a result, the keratinizing microorganisms growing in such conditions correspond to the trends of sustainable development. Moreover, unlike chemical hydrolysis, no harsh substances are used during the process of biodegradation (Shestakova et al., 2021).

Biodegradation of chicken feathers has many advantages including cost-effectiveness, preservation of essential amino acids, moreover; it can be applicable in the production of animal feed and fertilizer in addition to its advantage as an eco-friendly approach (Javeed et al., 2020). The present study concerned with measuring the production of soluble protein and free amino acids in the fermented media, produced by the activity of keratinolytic fungi. Maximum level of soluble protein 486 µg/ml was achieved by the activity of *Aspergillus niger*. As for Lasekan et al. (2013) it is very important to develop biotechnological methods for processing feathers, with the aim of further valuing this abundant and low-cost protein-rich biomass.

Researchers are interested in using keratin-degrading microorganisms to clean feathers as they can completely hydrolyze keratin under minor conditions and produce better hydrolysate rich in amino acids and peptides (Schor & Krimm, 1961; Brandelli et al., 2015; Qiu et al., 2020). Hydrolysates containing amino acids and peptides may be directly absorbed by plant roots and leaves, where they may be transmitted to other plant tissues and eventually act as growth stimulants (Colla et al., 2015). Feather hydrolysates are showing promising results for growing ryegrass, wheat, rice, Bengal gram, beans and vegetables. The application of hydrolysates produced from keratin-

rich materials, increased the germination of seed, root length, stem and root weight, and also enhanced productivity (Callegaro et al., 2019**)**.

Keratinases can be added to animal feed as additives to help break down proteins, which will increase animal weight and aid in better digestion. Recently, it was discovered that feather meal treated with the compound enzymatic hydrolysis (CEH) from *B.amyloliquefaciens* 3-2 contained more free amino acids and soluble peptides. Additionally, the in vitro digestibility and protein solubility both rose by 10.27 and 20.75 times, respectively. These results indicate that improving the nutritional value of feather waste by CEH may be a promising strategy (Zhou et al., 2020). The significant degradation of chicken feather waste which present in chicken feather medium was an indication that the fungal species showed potentials as industrially important organisms.

CONCLUSION

Keratinolytic fungi are important ecologically because they are involved in keratin degradation. Consequently, a collection of fungal strains were isolated from dumpsites using chicken feather medium as the only carbon and nitrogen source. Our research resulted four fungal species belong to *Aspergillus* genera, which have the ability to produces degraded enzymes that can break down complex keratinous substrates in nature, hence they are responsible for the biodegradation of keratinous material in polluted areas. In addition to studying the ability to produce free amino acids in the fermented medium. The degradation of chicken feather wastes into valuable product using microbial-based technology represents sustainable development from both economic and environmental perspectives.

REFERENCES

Bagewadi, Z. K., S. I. Mulla and H. Z. Ninnekar (2018). "Response surface methodology based optimization of keratinase production from Trichoderma harzianum isolate HZN12 using chicken feather waste and its application in dehairing of hide." Journal of Environmental Chemical Engineering **6**(4): 4828-4839.

Belmessikh, A., H. Boukhalfa, A. Mechakra-Maza, Z. Gheribi-Aoulmi and A. Amrane (2013). "Statistical optimization of culture medium for neutral protease production by Aspergillus oryzae. Comparative study between solid and submerged fermentations on tomato pomace." Journal of the Taiwan Institute of Chemical Engineers **44**(3): 377-385.

Bhari, R., M. Kaur and R. Sarup Singh (2021). "Chicken feather waste hydrolysate as a superior biofertilizer in agroindustry." Current Microbiology **78**(6): 2212-2230.

Brandelli, A., L. Sala and S. J. Kalil (2015). "Microbial enzymes for bioconversion of poultry waste into added-value products." Food Research International **73**: 3-12.

Callegaro, K., A. Brandelli and D. J. Daroit (2019). "Beyond plucking: feathers bioprocessing into valuable protein hydrolysates." Waste Management **95**: 399-415.

Colla, G., S. Nardi, M. Cardarelli, A. Ertani, L. Lucini, R. Canaguier and Y. Rouphael (2015). "Protein hydrolysates as biostimulants in horticulture." Scientia Horticulturae **196**: 28-38.

Elkhwesky, Z. (2022). "A systematic and major review of proactive environmental strategies in hospitality and tourism: Looking back for moving forward." Business Strategy and the Environment. https://doi.org/10.1002/bse.3076

Elkhwesky, Z., Y. El Manzani and I. E. Salem (2022). "Driving hospitality and tourism to foster sustainable innovation: A systematic review of COVID-19-related studies and practical implications in the digital era." Tourism and Hospitality Research. https://doi.org/10.1177/14673584221126792

Elkhwesky, Z. and E. F. Y. Elkhwesky (2022). "A systematic and critical review of Internet of Things in contemporary hospitality: a roadmap and avenues for future research." International Journal of Contemporary Hospitality Management(ahead-of-print). https://doi.org/10.1108/IJCHM-01-2022-0090

Elkhwesky, Z., I. E. Salem, M. Varmus and H. Ramkissoon (2022). "Sustainable practices in hospitality pre and amid COVID-19 pandemic: Looking back for moving forward post-COVID-19." Sustainable Development. https://doi.org/10.1002/sd.2304

Fagbemi, O. D., B. Sithole and T. Tesfaye (2020). "Optimization of keratin protein extraction from waste chicken feathers using hybrid pre-treatment techniques." Sustainable Chemistry and Pharmacy **17**: 100267.

García, J. A. C., J. M. R. Pino, Z. Elkhwesky and I. E. Salem (2022). "Identifying core “responsible leadership” practices for SME restaurants." International Journal of Contemporary Hospitality Management(ahead-of-print). https://doi.org/10.1108/IJCHM-09-2021-1194

Gupta, R., R. Rajput, R. Sharma and N. Gupta (2013). "Biotechnological applications and prospective market of microbial keratinases." Applied microbiology and biotechnology **97**(23): 9931-9940.

Gupta, R. and P. Ramnani (2006). "Microbial keratinases and their prospective applications: an overview." Applied microbiology and biotechnology **70**(1): 21-33.

Isaac, G. S. and M. A. Abu-Tahon (2016). "Dehairing capability of alkaline keratinase produced by new isolated Cochliobolus hawaiiensis AUMC 8606 grown on chicken feather." Romanian Biotechnological Letters **22**(6): 12147-12154.

Javeed, F., M. G. Shahid and A. Javed (2020). "Effect of Different Operational Parameters on Bio-degradation of Chicken Feathers by Aspergillus niger: Investigation Under Submerged Fermentation Process: Parameters on Bio-degradation of Chicken Feathers." Biological Sciences-PJSIR **63**(2): 71-76.

Kim, J.-D. (2003). "Keratinolytic activity of five Aspergillus species isolated from poultry farming soil in Korea." Mycobiology **31**(3): 157-161.

Lasekan, A., F. A. Bakar and D. Hashim (2013). "Potential of chicken by-products as sources of useful biological resources." Waste management **33**(3): 552-565.

Mazotto, A. M., S. Couri, M. C. Damaso and A. B. Vermelho (2013). "Degradation of feather waste by Aspergillus niger keratinases: comparison of submerged and solid-state fermentation." International Biodeterioration & Biodegradation **85**: 189-195.

Qiu, J., C. Wilkens, K. Barrett and A. S. Meyer (2020). "Microbial enzymes catalyzing keratin degradation: Classification, structure, function." Biotechnology Advances **44**: 107607.

Schor, R. and S. Krimm (1961). "Studies on the structure of feather keratin: II. A β-helix model for the structure of feather keratin." Biophysical Journal **1**(6): 489-515.

Shaaban, M., M. M. El-Metwally, A. A. Mekawey, A. B. Abdelwahab and M. M. Soltan (2022). "Monascin and monascinol, azaphilonoid pigments from Mortierella polycephala AM1: in silico and in vitro targeting of the angiogenic VEGFR2 kinase." Zeitschrift für Naturforschung C **77**(1-2): 11-19.

Sharma, R. and S. Devi (2018). "Versatility and commercial status of microbial keratinases: a review." Reviews in Environmental Science and Bio/Technology **17**(1): 19-45.

Shestakova, A., S. Timorshina and A. Osmolovskiy (2021). "Biodegradation of Keratin-Rich Husbandry Waste as a Path to Sustainable Agriculture." Sustainability **13**(16): 8691.

Tamreihao, K., S. Mukherjee, R. Khunjamayum, L. J. Devi, R. S. Asem and D. S. Ningthoujam (2019). "Feather degradation by keratinolytic bacteria and biofertilizing potential for sustainable agricultural production." Journal of basic microbiology **59**(1): 4-13.

Tesfaye, T., B. Sithole and D. Ramjugernath (2017). "Valorisation of chicken feathers: a review on recycling and recovery route—current status and future prospects." Clean Technologies and Environmental Policy **19**(10): 2363-2378.

Verma, A., H. Singh, S. Anwar, A. Chattopadhyay, K. K. Tiwari, S. Kaur and G. S. Dhilon (2017). "Microbial keratinases: industrial enzymes with waste management potential." Critical reviews in biotechnology **37**(4): 476-491.

Vidmar, B. and M. Vodovnik (2018). "Mikrobne keratinaze: enzimi s obećavajućom biotehnološkom primjenom." Food Technology and Biotechnology **56**(3): 312-328.

Wang, B., W. Yang, J. McKittrick and M. A. Meyers (2016). "Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration." Progress in Materials Science **76**: 229-318.

Zhou, L., X. Xie, T. Wu, M. Chen, Q. Yao, H. Zhu and W. Zou (2020). "Compound enzymatic hydrolysis of feather waste to improve the nutritional value." Biomass Conversion and Biorefinery: 1-12.

J. Agric. & Env. Sci. (Damanhour University) 2022, 21(2): 1-18
Print: ISSN 1687-1464 Online: 2735-5098

الملخص العربي

الفطريات المحللة للريش: عزل وتحديد وقياس النشاط البروتيني بإستخدام تقنية تخمير الحالة الصلبة

نعمه درهاب[١]*-محمد مجدي المتولي[١] - مني السيد مبروك[١] –يوسف مصطفي محمد[١]

[١]قسم النبات والأحياء الدقيقة، كلية العلوم، جامعة دمنهور، دمنهور، مصر

***Corresponding author:** Email: neama844@sci.dmu.edu.eg
***ORCID**: https://orcid.org/0000-0001-8901-4289

الملخص

يؤدي النمو السريع لصناعة الدواجن الي زيادة إنتاج المخلفات المحتوية على الكيراتين (ويعتبر الريش أكثر المصادر الكيراتينية الموجوده في البيئه). لذلك أصبحت الكائنات الميكروبية التي لها القدرة علي تكسير الكيراتين مهمة في التقنية الحيوية لأنها تستهدف التحلل المائي للكيراتين شديد الصلابة. الهدف من هذه الدراسة هو الحصول على عزلات فطرية ذات القدرة علي النمو وتكسير الكيراتين. حصلت دراستنا على أربع عزلات فطرية كيراتينية تم جمعها من ثلاث عينات من أماكن مختلفة. وذلك بإستخدام وسط تخمير الحالة الصلبة (SSF) الذي يحتوي على الريش كمصدر وحيد للكربون والنيتروجين. وبعد التنقية بإستخدام وسط غذائي (PDA)؛ والتعرف على العزلات من خلال دراسة الخصائص المزرعية والخصائص المجهرية؛ تم اعتبار العزلات الفطرية بالأسماء الاتية؛ *Aspergillus terreus* و *Aspergillus nidulans* و *Aspergillus niger* و *Aspergillus fumigatus*. تم تطبيق هذه الكائنات علي وسط تخمر يحتوي علي الريش وتحضينه لمدة ست أيام ثم بعد ذالك تم قياس كمية الأحماض الأمينية والبروتينات الذائبة الناتجه عن نشاط الفطريات في تكسير الريش الصلب. ويمكن أن نستفيد من هذه الأنواع بإستخدامها وتطبيقها في مجال التكنولوجيا الحيوية، مثل إدارة ومعالجة النفايات الكيراتينية.