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Full Length Article

Proteolytic Activity and Health Impact of Fungi Isolated from Chickens Feathers in House Breeding Cages

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Abstract

The vast majority of fungi are saprophytic and have the ability to utilize dead plant and animal tissues. Keratinophilic fungi are a specific group that decomposed keratinized structures of humans and animals viz skin, hair, feathers, etc. and may cause mycoses as opportunistic or true pathogens. The house breeding cages of chickens are a source of saprophytic fungi including keratinophilic group. The present study aims to isolate fungi from feathers in house breeding cages, explain the composition of the fungal community, examine the proteolytic activity of fungal taxa via skimmed milk agar test, as well as clarify their health risk on breeders. A total of fifty feather samples were collected from urban sites in Erbil city. Samples were cultured on Sabouraud's dextrose agar and Typha pollen agar. Petri dishes were incubated at 25ºC and the developed fungi were checked after three days and for four weeks. Eighteen fungi were recorded belonging to 14 genera viz *Aspergillus* (5 species), *Chrysosporium* sp., *Fusarium* sp., *Chaetomium* sp., *Emericella* sp., *Papulospora* sp., *Cunninghamella* sp., *Rhodotorula* sp., *Candida* sp., *Acremonium* sp., *Cladosporium* sp., *Mucor* sp., *Myceliophthora* sp., and *Phoma* sp. Aspergilli exhibited the highest occurrence%=100% and frequency%34.16 followed by *Chrysosporium* sp. with (90 and 20.8% respectively). 6 isolates out of 18 (30%) represented remarkable proteolytic activity, the higher proteolytic activity via enzymatic index (EI) showed that the isolates *Myceliophthora* sp., *Acremonium* sp., *Chaetomium* sp., and *Chrysosporium* sp. were (EI =1.75; 1.5; 0.928 and 0.827 respectively). ANOVA test indicated that there were no significant differences between the examining isolates. It is worth mentioning that all isolated genera were reported as opportunistic pathogens and are responsible for several fungal infections viz allergy, dermatomycosis, and deep infections.

Keywords: Poultry; Proteolytic; Feathers; Keratinase; Health impact; Keratinophilic fungi

Introduction

Feathers were described as a carrier of fungi that can infect other animals and humans when prompting factors are present. The structure of feathers is compos of dead keratin-filled corneocytes (Camin *et al.* 1998; Wang *et al*. 2016). Generally, keratinized tissues are not easily biodegradable in the environment. A specific ecological group of fungi has the ability to utilize keratinized tissues. and decompose them into simple primary components (Călin *et al*. 2017).

Keratinophilic fungi inhabit feathers and featherless sites of birds (Deshmukh 2004). This group can also infect humans causing types of diseases viz superficial, cutaneous, and onychomycosis, as well as pulmonary besides several types of infections in poultry and domestic animals. Fungi associated with feathers are one of the means to get rid of feather waste at the level of the poultry industry. It is worth mentioning that biodegradation is the best method to deal with the huge feathers waste in poultry farms and looking for active proteolytic fungi is the goal of several studies carried out to get rid of these pollutants (Kumar and Kushwala 2014). Fungi on feathers were studied by several workers around the world. Studies that described the fungal population of feathers showed a high fungal diversity. In Tuscany /northwest of Italia Nardoni and Mancianti (2021) identified 11 Keratinophilic fungi from feathers of aquatic and terrestrial birds viz *Scopulariopsis brevicaulis, Chrysosporium keratinophilum, Trichophyton terrestre, Microsporum gypseum, Sepedonium* sp., *Chrysosporium pannorum, Myriodontium* sp., *Chrysosporium tropicum, Chrysosporium pruinosum, Chrysosporium luteum,* and *Aphanoascus fulvescens*. From 4 types of birds, 12 keratinophilic fungi and 2 dermatophytes viz *Microsporum*, and *Trichophyton* were identified in Nigeria by Efuntoye and Fashanu (2002). *Chrysosporium* (3 sp.) showed the highest incidence. Feathers of 43 species of bird pets were examined in Erbil city/north of Iraq, 14 saprophytic fungi, 8 keratinophilic and 3 dermatophytes were identified (Mohammed *et al*. 2017).

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Several studies focused on the other side of view, they distinguished fungi transmitted locally by bird's feathers or globally during the migration season, these including high human pathogens and livestock zoonosis infections (Sotirios *et al.* 2008; Akter *et al*. 2020; Cabe 2021). Litter in poultry houses was examined also and the incidence of keratinophilic and toxigenic fungi occurrence was recorded (Viegas *et al.* 2012).

In Iraq, keratinophilic fungi were isolated and identified in soil, sediments, birds litters and water. The indoor and outdoor habitats were examined for keratinophilic fungi also (Abdullah and Hassan 1995; Abdullah and Abbas 2008; Al-Mosa and Abdullah 2011; Imran and Ali 2014; Mohammad and Habeb 2016; Hussein and Abdullah 2017). Unfortunately, there is a lack of information on keratinophilic fungal groups associated with feathers, Mohammed *et al.* (2017) collected feather samples from 43 types of birds and identified the saprophytic, dermatophytes and yeasts. A study to identify fungi of poultry feed and their capability to produce mycotoxins has been carried out by Sharee (2010), and Aspergilloses outbreaks in commercial broiler chickens (Eassa *et al*. 2017).

The aim of this study was to investigate fungal communities associated with chicken feathers in house breeding cages, examine the proteolytic activity of the identifying fungi and discuss their health hazards on breeders.

Materials and Methods

Sample collection

Fifty feather samples were collected in December 2022 from several urban locations in Erbil city. All samples were without dropping or moistened soil materials, which were kept separately in nylon bags at 4ºC.

Culturing and identification

Three types of culture media were used, Sabouraud's dextrose agar (SDA=61gm/L) and the lab-made Typha pollen agar (TPA=5gm/L) (Al-Bader 2018a) for the primary culturing, as well as diluted skim milk agar (SMA=20 gm/L) for proteolytic activity test. The culture media were supplemented by Chloramphenicol (15 mg/L) to prevent bacterial growth. The direct plate method was followed, and pieces of feathers were fixed on the surface of the culture media by sterile forceps. The Petri-dish plates then were incubated at 25± 2.

The cultures were checked daily from the third day over a period of 4 weeks. The observed developing fungal growths were directly transferred to Sabouraud's dextrose agar to prepare a pure culture. The identification was conducted via macroscopic and microscopic characteristics based on (Domsch *et al.* 1989; De Hoog and Guarro 2001; Aravinitis and Mylonakis 2015).

Fungal community analysis

In order to identify the predominant taxa, the occurrence% and frequency % was calculated via the following equations: O% = (No. of times fungal appear /No. of collected samples) X100 F%= (No. of fungal isolates/No. of total fungal isolates) X100 (Al-Bader and Zefenkey 2023).

Detection of protease activity

The proteolytic activity was examined for the isolated fungi by using the diluted skim milk agar medium -SMA- (Kanchana 2013). Petri plates with 2% skim milk agar were prepared and inoculated with active growth from the edge of 7 days of pure fungal cultures. Plates were incubated at 25±2°C and after 4 days, the clear zone around colony growth was observed and measured. The mean diameter was measured in two perpendicular directions, the mean of three replicates was calculated, and the Enzymatic Index (EI) has been calculated according to the equation: Diameter of clear zone-diameter of growth/diameter of the growth. (Lechuga *et al*. 2016).

Results

The fungal community structure: A total of 298 isolates were counted from fifty feather samples, these belong to fourteen fungal genera. The identified taxa included 5 species of *Aspergillus* viz *A. fumigatus, A. flavus, A. nidulans, A. niger*, *A. parasiticus* as well as 13 genera belonging to several taxonomic groups including *Acremonium hyalinum, Cladosporium cladosporoidis, Chrysosporium* sp., *Myceliophthora* sp., *Papulospora* sp., *Fusarium* sp., *Mucor miehie, Cunninghamella elegans, Emericella nidulans, Chaetomium* sp., *Candida* sp., *Rhodotorula mucilagenosa* and *Phoma herbarum.*

Aspergillus spp. occurred in all feather samples (O=100%) followed by *Chrysosporium* sp. (O=90%) (Table 1).

Hyphomycetes represented the highest occurrence (46 samples, 92%), followed by Zygomycetes (10samples, 20%), Basidiomycetes (6 samples, 12%), Ascomycetes and Coelomycetes (4 samples, 8%) (Fig. 1).

The biodegradable activity of isolated fungi: Results of the SMA test showed that only 6 taxa exhibited clearing zones (Table 2 and Fig. 2), the tested isolates exhibited deformed colonies on diluted skimmed milk agar except for *Aspergillus flavus* which showed normal macroscopic characteristics.

Fig. 2 illustrates that *Myceliophthora* sp. (no.4), *Acremonium hyalinum* (no.5), showed the highest EI followed by *Chaetomium* sp. (no.2), *Chrysosporium* sp. (no. 3). *Papulospora* sp. (no.6) and *A. flavus* (no.1) proved weak proteolytic activity. The remaining fungal taxa showed weak proteolytic activity with narrow zones (<3 mm) and were not easy to measure.

Table 1: The isolated fungi with occurrence% (O%) and frequency % (F%). H=Hyphomycetes, A=Ascomycetes, Z=Zygomycetes, C= Coelomycetes, B=Basidiomycetes

Fungi		T.G.	No. of isolates	O%	F%
1	*Acremonium hyalinum*	H	16	26	5.3
2	*Aspergillus flavus*	H	29	50	9.73
3	*A. fumigatus*	H	19	28	6.37
4	*A. nidulans*	H	10	14	3.33
5	*A. niger*	H	29	44	9.73
6	*A. parasiticus*	H	15	22	5.03
7	*Candida sp.*	A	10	14	3.35
8	*Chaetomium sp.*	A	8	4	2.68
9	*Chrysosporium sp.*	H	62	90	20.8
10	*Cladosporium cladosporioides*	H	29	46	9.73
11	*Cunninghamella elegans*	Z	4	8	1.34
12	*Emericella nidulans*	A	6	12	2.01
13	*Fusarium sp.*	H	19	34	6.37
14	*Mucor miehei*	Z	14	24	4.6
15	*Myceliophthora sp.*	H	8	16	2.69
16	*Papulospora sp.*	H	3	4	1.00
17	*Phoma herbarum*	C	8	16	2.68
18	*Rhodotorula mucilaginosa*	B	9	10	3.02

Table 2: Enzymatic index (EI) of the tested isolates. The results are means of 3 replicates

No.	Fungi	CZD/mm	GZD/mm	EI
1	*Myceliophthora sp.*	33	12	1.75
2	*Acremonium sp.*	35	14	1.5
3	*Chaetomium sp*	27	14	0.928
4	*Chrysosporium sp.*	52	28	0.857
5	*Papulospora sp.*	46	34	0.352
6	*Aspergillus flavus*	27	14	0.08

CZD= clear zone diameter(mm), GZD=growth zone diameter

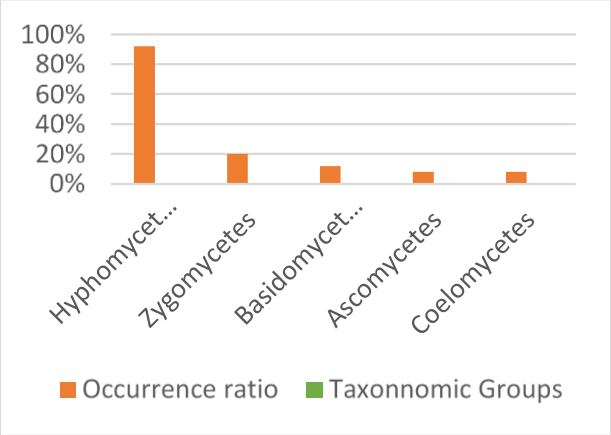

Fig. 1: The taxonomic groups % of isolated genera

Discussion

Feathers showed high fungal diversity which is a reflection of suitable environment for fungal growth. Moorthy *et al.* (2011) reported that feather structure was more attractive to keratinophilic fungi than other keratinous tissues. The identified fungi here were commonly recorded in previous studies on keratinophilic fungi associated with feathers, as well as poultry environments and other keratinous tissues (Kim 2003; Mandeel *et al.* 2011; Al-Bader 2018b). The predominance of Hyphomycetes in the present study is in agreement with studies of keratinophilic fungi in different keratinous tissues and soil habitats (Deshmukh 2004; Mandeel *et al.* 2011; Singh 2022).

The four species of Aspergillus besides Chrysosporium, Cladosporium and Fusarium which represented the higher incidence in the current study were commonly recorded as keratinophilc fungi. This group also was frequently isolated from the soil via keratinous baits (Al-Mosa and Abdullah 2011; Kumar and Yadav 2020; Zhang *et al*. 2021). Searching for active and safe processes to get rid of great amount of feather's waste encourage studies on fungi associated with feathers. The use of eco-friendly processes in the biodegradation of poultry waste is the goal of several works and it was supported by health agencies. Studies suggested using active proteolytic fungi instead landfilling or burning since these isolates are eco-friendly agents and create useful organic products for agricultural use (Kumar and Kushwaha 2021).

Aspergillus spp. which was predominant in the current study is a cosmopolitan fungus, it has diverse extracellular enzymes that enable it to colonize feathers and different substrates including feed, soil and wastes (Kim 2003; Mandeel *et al*. 2011; Imran and Ali 2014; Abdullah and Al-Bader 1990; Alkhursan *et al.* 2021). Snyman *et al*. (2019) explained that Aspergillus extracellular keratinase activity refers to protease gene expression. It is worth mentioning that Aspergillus is used in feathers waste biodegradation (Bhari *et al.* 2021; Aina *et al*. 2021).

Chrysosporium sp. the second predominant isolate from tested samples, is a large genus of the saprophytic life mode, it is widely distributed and can inhabit several types of substrates in a wide range of temperature and humidity levels (Kumar *et al*. 2020). *Chrysosporiu* is commonly found in soils, air and the surface bodies of animals (Gurung *et al*. 2018). The adaptation of *Chrysosporium* to various environmental factors is related to its capacity to produce different and unique enzymes and secondary metabolites (Han *et al*. 2017). *Chrysosporium* sp. The fungus showed remarkable proteolytic activity in the present study and it was pointed out by several researchers as a good choice for feather waste management (Kumar and Kushwala 2014; Koutb *et al.* 2023).

Cladosporium is cosmopolitan in distribution and commonly encountered on all kinds of substrates including leather-made objects. Nwadiaro *et al*. (2015) explained the biodegradation activity of Cladosporium and selected isolates were used in biotechnology processes especially leathers manufacture (Călin *et al.* 2017).

Fusarium spp. was regarded initially as a plant pathogen while studies in later years mentioned it as keratin inhabiting fungus, which can be isolated from the soil via keratin baits (Hamm *et al*. 2020). The fungus showed a wide range of animal infections including feathers and other keratinous tissues (Preczeski *et al*. 2020; Kumar and Kushwaha 2021).

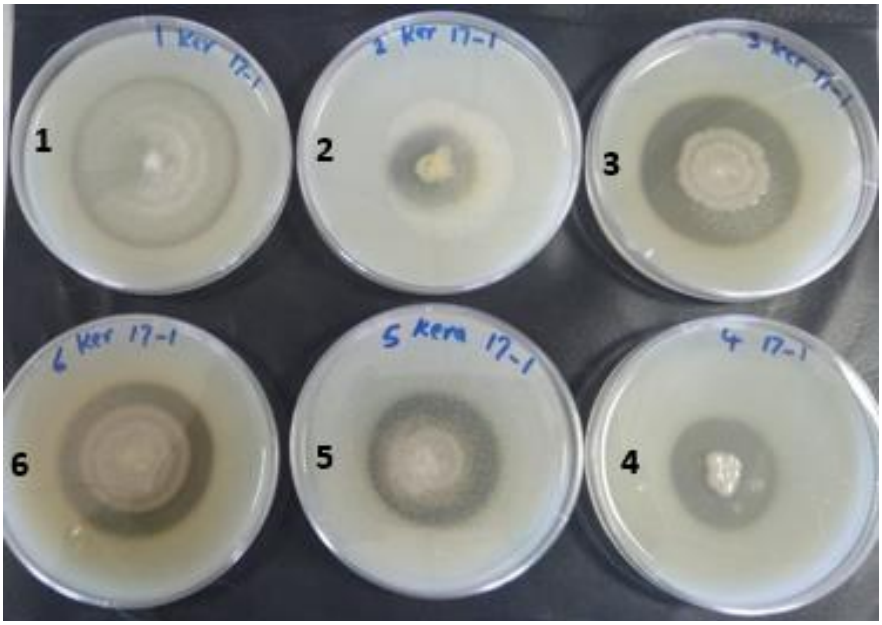

Fig. 2: The proteolytic activity (clear zone) by SMA culture medium. *Asp. flavus* (no.1). *Chaetomium* sp. (no.2), *Chrysosporium* sp. (no.3), *Myceliophthora* sp. (no.4), *Acremonium hyalinum* (no.5) and *Papulospora* sp.(no.6). There is no significant differences between tested isolates as revealed from analysis of variance (Table 3).

Table 3: Analysis of variance for proteolytic activity between and within the groups of fungi

Source of variation	Degree of freedom	Sum of square	Mean square	F-stat	P-value
Between groups	5	580.7836	116.1567	0.3309	0.8847
Within groups	12	4212.4987	351.0416	-	-
Total	17	4793.2823	-	-	-

Even though *Myceliophthora* sp. had a low incidence level in the present study, the isolate represented the highest enzyme index for proteolytic activity. Myceliophthora has a well-known keratinophilic species including *M. veellerea* which was isolated from soil (Singh and Kushwaha 2010), and it was reported as the predominance keratinophilic genus in Mosul forest soil (Abdullah and Al-Bader 1997). *M. verrucosa* also was reported as an active proteolytic species, it has been ascertained by the increased damage in feathers with an abundance of the fungus (Baho 2022).

Cladosporium sp. isolated from soil showed high keratinolytic activity and it was suggested as a biodegradable eco-friendly agent for keratin waste materials in several studies (Călin *et al*. 2017; Constantin *et al*. 2022).

Acremonium and *Mucor* showed moderate abundance, both were commonly isolated from feather samples (Singh and Kushwaha 2010).

The remaining genera with low abundance can hydrolyze keratin and several organic substrates. These have been recorded in several studies as a part of the feather's fungal community or associated with other keratinous tissues. (Najwa and Abu-Mejdad 2013; Mohammed *et al*. 2017; Al-Bader 2018a,b; Kumar and Kushwaha 2021).

The health hazards of isolated fungi/ Even though pathogenicity is a trait of species or strain, the keratinophilic genera include dermatophytes and opportunistic taxa and are probably agents of diseases (Torres-Rodríguez and López-Jodra 2000) and particular attention should be taken to fungi that colonize feathers and chicken house environment. Several researchers discussed and warned about the health problems of a feather–associated fungi and the current results showed that feathers are a rich source of opportunistic fungi, and their hazard increased because of producing a high number of easily aerosolized conidia. These airborne structures are a probable source of respiratory disorders, superficial, cutaneous, systemic mycoses, otitis, dermatomycosis, etc. (De Hoog and Guarro 2001).

Aspergillus spp. is the predominant isolate in the present study, it is a worldwide genus, it has a well-known saprophytic nutrition mode and it consists of several species that can cause infections in healthy individuals. Aspergillus produces a great number of fine conidia that are easily dispersed as aerosolized and are easily inhaled, so *Aspergillus* is among the most air mycobiota and has high relation with pulmonary disorders (Wang *et al*. 2022). Furthermore, *Aspergillus spp.* are wound-contaminated and toxin producer fungi (Mousa *et al*. 1999; Tosti *et al*. 2000). The high number of dry conidia of *Aspergillus* which are easily aerosolized increases their health hazards, especially for pulmonary infections. *Aspergillus fumigatus* is the major cause of

invasive aspergillosis (Bart-Delabesse and Latge 2004; Liu *et al.* 2021). *A. flavus* is one of the most important species that can cause both noninvasive and invasive systematic aspergillosis in immunocompromised individuals (Hatmaker *et al.* 2022). The predominant airborne species, *A. niger*, is the main etiologic agent of otitis (Roohi *et al*. 2023).

Chrysosporium sp. The fungus has a high activity to utilize keratinous tissues and over the past 20 years, it was recorded as an emerging pathogen. The *Chrysosporium* cutaneous infection may be fatal in types of reptiles (Cabañes *et al*. 2014). Species of *Chrysosporium* are isolated from skin and nail scrap samples, especially from feet and a rare subcutaneous infection. (Mijiti *et al*. 2017). The moderate proteolytic activity of *Chrysosporim* sp. in the present study may be due that protease activity is a strain-dependent characteristic (Griffiths *et al*. 2018).

Cladosporium which occurred in the third level here, is well-known with worldwide distribution. It is commonly isolated from soil and organic matter. It represents among the most frequently culturable airborne fungi. The genus includes true human-pathogenic species (Bensch *et al*. 2012). Long-term exposure to a large amount of *Cladosporium* propagules can cause adverse health effects, including allergies and asthma symptoms, as well as infections of the eye, ear, sinus, and skin problems as well as phaeohyphmycosis (Ogórek *et al.* 2012; Sandoval-Denis *et al.* 2016).

Fusarium is the causative agent of Fusariosis, the infection of plants, animals, and humans caused by various fungi of the genus. Human infections are not easily treated, this may be related to the antifungal resistance of several environmental strains (Ribas *et al.* 2016). Most infections in humans range from superficial and locally invasive to distributed, with the most common infections being onychomycosis, skin infections and keratitis (van Diepeningen *et al*. 2015; Preczeski *et al.* 2020).

Mucor contains about 50 taxa, they are widespread on decaying food, soil and animal excrement. Taxa with health importance include a few thermotolerant species which are the agents of several types of infections such as pulmonary mucormycosis, which develops after inhalation of spores into the bronchioles and alveoli (Agrawal *et al*. 2020).

The other taxa in (Table 1) have been reported as agents of several types of mycoses ranging from superficial to deep infections. The health risks of *Acremoium* sp., *Candida* sp., *Chaetomium* sp., *Cunninghamella* sp., *Emericella* sp., *Myceliophthora* sp., *Paulospora* sp., *Phoma* sp., and *Rhodotorula* sp. increase remarkably for immunodepleted individuals and with exposure time (Das *et al*. 2010; Selvin *et al*. 2014; Talapko *et al*. 2021; Cronin *et al*. 2021; Hallur *et al*. 2021; Rai *et al.* 2021; Kim *et al*. 2021; Baho 2022).

Conclusion

Depending on the findings, the fungal community on feathers has the same composition that occurred on other keratinous substrates in the same study region as well as keratinophilic fungi of soil. The results showed that a third of isolated genera had remarkable proteolytic activity, and these fungi have an important role in the feather's biodegradation in nature. Fungi in the feathers were recorded as hazardous agents for chicken breeders, which is worse for children and oldsters and individuals who are interested in this subject must follow health regulations to prevent infections.

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Author Contributions

This is contribution from the sole author, Salah Mahdi Al-Bader

Conflicts of Interest

No conflicts of interest

Data Availability

Including in the text

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