



Morphological Characteristics of Fungi Species Isolated From Dairy Products in Fayoum Governorate



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EIGHTY samples of different dairy products were collected from different markets in Fayoum Governorate. Samples were examined for incidence of filamentous fungi. Thirty five isolates of fungi were isolated and morphologically identified. The identification of fungi isolates mainly depended on colony characteristics (color and texture) and microscopic appearance including shape and branching of conidiophores, presence or absence of metulae, shape of phialides and texture of conidia. Filamentous fungi were isolated on Sabouraud Dextrose Agar (SDA) medium. Czapek Yeast Extract Agar (CYA) medium was used for the morphological identification of isolated fungi colonies. Lactophenol cotton blue dye was used to stain the microscopic slides of fungi species to prepare to microscopic examination. Then the microscopic images were taken by Canon G6 digital camera at a microscopic magnification power 1000x. The results revealed that all isolates fell into five fungi species classified into (four species of *Aspergillus terreus*, nine of *Aspergillus niger*, nine of *Aspergillus flavus*, eight of *Aspergillus parasiticus*, and five of *Penicillium corylophilum*).

Keywords: Fungi, *Aspergillus*, *Penicillium*, Morphological characterization.

Introduction

Fungi play an important role in dairy products. Since fungi mostly cause organoleptic changes, desirable in some circumstances, but they can be harmful in other instances because of the production of secondary metabolites such as mycotoxins. However, fungal contamination causes several undesirable organoleptic changes such as: gas production, off-flavors, off-odors, proteolysis, and lipolysis (Horwood et al. 1987; Vivier et al. 1994 and Maraz & Kovacs, 2014). Moreover, growth of moulds is a defect which occurs usually on surface of hard cheese and packaged cheese (Hocking and Faedo, 1992). Most moulds commonly found belonged to *Penicillium* and *Aspergillus* and were commonly spread contaminants of cheese (Gandomi et al. 2009).

However most *Aspergillus* species cause economical losses through poor appearance, off

flavours as well as public health hazards due to their secondary toxic metabolites. Some of these mycotoxins as Strigmat and Ocystin may cause liver cancer (Hemant et al. 2005).

Fungi, especially moulds, can be morphologically identified by determining colonial features and morphological structure (Asan A. 2004 and Mushimiyimana et al. 2016). Also, fungi are identified on the basis of gross cultural and microscopic characteristics described by Samson et al. (1998) and Pal (2007). Moreover, El-Fadaly et al. (2015) identified some fungal isolates isolated from Ras cheese in Egypt according to morphological characteristics of colonies in different cultivation media.

The objective of this research has been focused on the isolation and identification of filamentous fungi present in some dairy products collected from Fayoum governorate, Egypt.

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Materials and Methods

Materials

Sample collection

Eighty samples of some dairy products (hard and soft cheese, market yoghurt, buttermilk, butter, cream and mozzarella cheese) were collected from different markets in Fayoum Governorate.

Microbiological media

Sabouraud Dextrose Agar (SDA) consists of 5g peptone from meat; 5g peptone from casein; 40g glucose; 15g agar, were obtained from Merck company.

Czapek Yeast Extract Agar (CYA) consists of 30 g sucrose 5g yeast extract 1 g dipotassium hydrogen phosphate 0.3 g sodium nitrate 0.05g potassium chloride 0.05g magnesium sulphate 0.001g ferrous sulphate 0.001g zinc sulphate 0.001g copper sulphate 15.00 agar were obtained from Merck company.

Lactophenol cotton blue stain consists of 25g phenol crystals; 0.05g cotton blue; 25g lactic acid; 20g glycerol; distilled water to final volume 100 ml was prepared and used for staining fungal isolates for microscopic analysis.

Chemicals were obtained from Sigma and Merck companies and all the chemicals used for this study were analytical grade (A.R.).

Methods

Isolation of fungal elements

For preparing to experiments 10g of each dairy sample were aseptically withdrawn and mixed in a flask containing 90 ml of sterilized distilled water. 10 ml of tri-sodium citrate (20% w/v solution) was added to 10g of cheese samples before 80ml of sterilized distilled water was added. Plates were inoculated by 1ml of the previous dilution and

incubated at 25°C for 5 days. Filamentous fungi grown in separated colonies were isolated on SDA medium.

Morphological identification of Fungal isolates

Identification basically depended on the morphological characteristics of fungal isolates grown on CYA cultivation medium. Colony characteristics (mainly color and texture) and microscopic appearance including shape and branching of conidiophores, vesicle size, presence or absence of metulae, shape of phialides, texture and dimensions of conidia were used for identification of isolated fungi in Assiut University Moubasher Mycological Centre (AUMMC), Assiut, Egypt, according to Manga *et al.* (2014).

Microscopic examination

Microscopic slides were prepared using scotch tape preparation method (Larone, 1995) wherein the fungi adhering to cello tape flag were mounted in lactophenol cotton blue. All fungi isolates were microscopically examined using trinocular Carl Zeiss, Axiostar Plus microscope and the micrographs were taken by Canon G6 digital camera (7.1 megapixels, with a magnification power 1000x, made in Japan), according to Raper and Fennell (1965), Pitt (1979) and Domsch *et al.* (2007).

Results and Discussion

Isolation and identification of fungal elements

As shown in Table 1, 35 fungal isolates were isolated and morphologically identified. Five groups of isolates were obtained (four of *Aspergillus terreus* Thom, nine of *Aspergillus niger* van Tieghem, nine of *Aspergillus flavus* Link, eight of *Aspergillus parasiticus* Speare, and five of *Penicillium corylophilum* Dierckx).

TABLE 1. Identified fungal species isolated from some dairy products in Fayoum Governorate.

AUMMC No.	Isolates count	Isolates ratio %	Identification
14054	4	11.4	<i>Aspergillus terreus</i>
14055	9	25.7	<i>Aspergillus niger</i>
14056	9	25.7	<i>Aspergillus flavus</i>
14057	8	22.9	<i>Aspergillus parasiticus</i>
14058	5	14.3	<i>Penicillium corylophilum</i>

AUMMC: Assiut University Moubasher Mycological Centre

Morphological identification and microscopic examination of isolated fungal species

As illustrated from the previous obtained results in Table 1, 35 fungal species were identified and divided into five fungal species.

Aspergillus terreus

Four isolates out of thirty-five were identified as *Aspergillus terreus* according to their colony characteristics (mainly color and texture) and microscopic appearance. As shown from Fig. 1, *Aspergillus terreus* colony has smooth-like walls, is brownish in color with off-white edges and gets darker as it ages on CYA medium Balajee (2009). *Aspergillus terreus* has conidial heads that are compact, biserial, and densely columnar. Conidiophores of *A. terreus* are smooth and hyaline. The conidia of *A. terreus* are small, about 2 μm in diameter, globose-shaped, smooth-

walled and can vary from light yellow to hyaline (Bizukoje and Ledakowicz, 2010).

Aspergillus niger

Nine isolates out of thirty-five were identified as *Aspergillus niger* according to their colony characteristics (mainly color and texture) and microscopic appearance. Figure 2 showed that *Aspergillus niger* colony is always starting white then quickly becoming black with conidial production. Hyphae are septate and hyaline. Conidial heads are splitting into columns. Conidiophores are long (400-3000 μm), smooth, and hyaline, becoming darker at the apex and terminating in a globose vesicle (30-75 μm in diameter) that in agreement with Steinbach and Stevens (2003). Metulae and phialides cover the entire vesicle. Conidia are brown to black, very rough, globose.

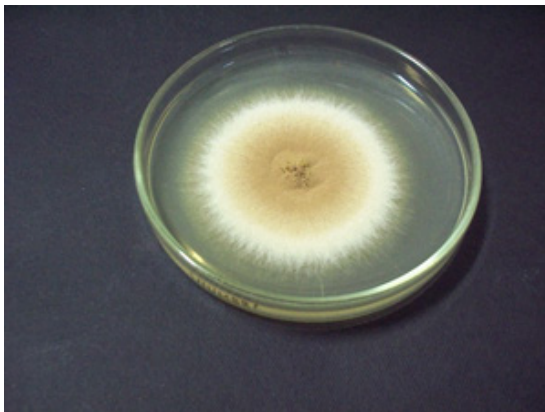


Fig. 1. *Aspergillus terreus* colony on CYA medium and its microscopic image.

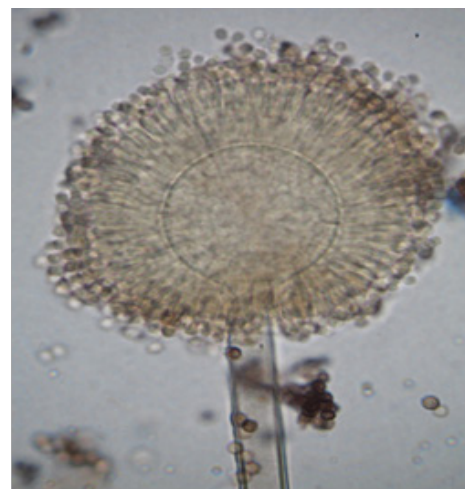
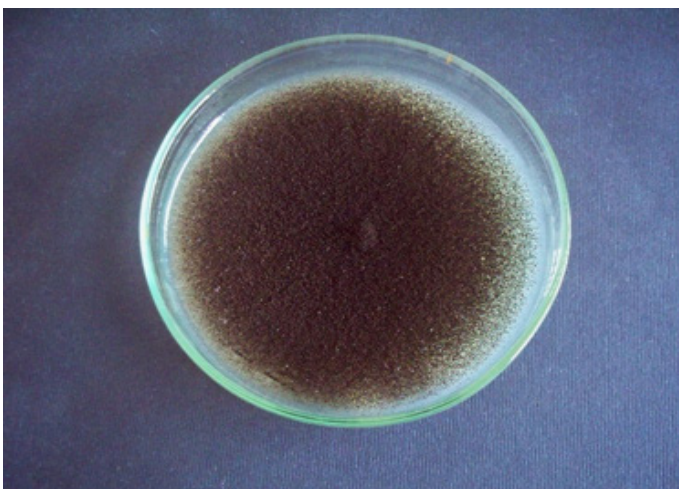


Fig. 2. *Aspergillus niger* colony on CYA medium and its microscopic image.

Aspergillus flavus

Nine strains of isolated fungal cultures were found to belong to *Aspergillus flavus* according to their colony characteristics (mainly color and texture) and microscopic appearance. It is clear from Fig. 3 that *Aspergillus flavus* colony had dark green conidia except the colony edge that was greenish-yellow. Moreover, the colony appeared downy or powdery in texture. Under microscope, *A. flavus* appeared to have radiating conidial heads. While the conidiophores appeared thick-walled and rough. The conidia were globose with thin wall. That were proved by Thathana *et al.* (2017).

Aspergillus parasiticus

Eight isolates out of thirty-five were identified as *Aspergillus parasiticus* according to their colony characteristics (mainly color and texture) and microscopic appearance. Figure 4 obviously showed that *Aspergillus parasiticus* colony was green as a color of olive with light green edge. The conidia of *A. parasiticus* had rough, thick walls and were spherical in shape. Conidial heads of *A. parasiticus* were dark green and unbranched. Conidia were distinctly roughened, globose to subglobose and are borne on stalks, which are commonly covered in small spines as described by Horn *et al.* (2009)

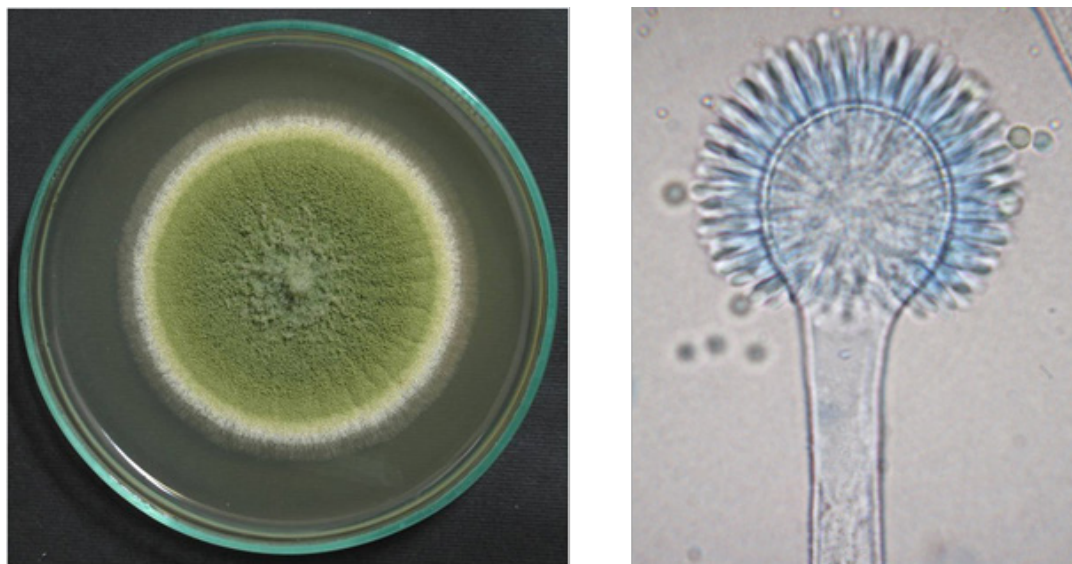


Fig. 3. *Aspergillus flavus* colony on CYA medium and its microscopic image.

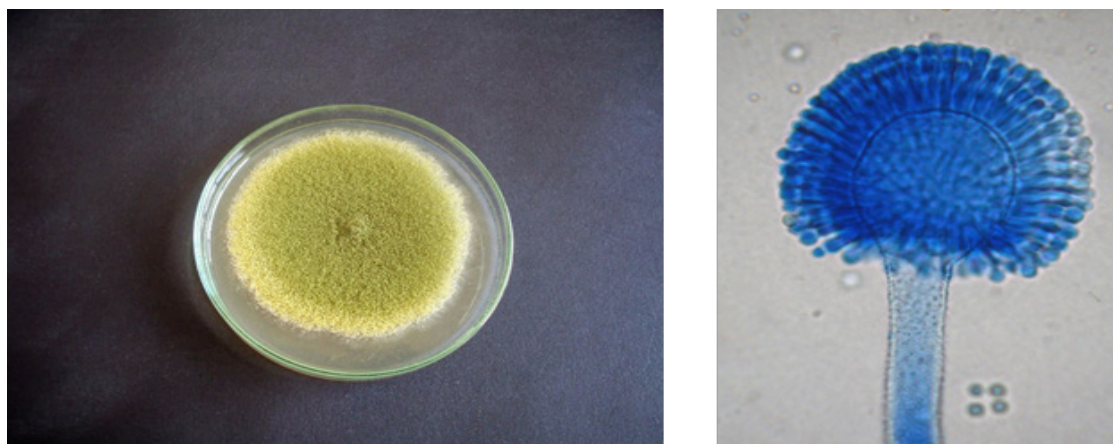


Fig. 4. *Aspergillus parasiticus* colony on CYA medium and its microscopic image.

Penicillium corylophilum

Five isolates out of thirty-five were identified as *Penicillium corylophilum* according to their colony characteristics (mainly color and texture) and microscopic appearance. It is obviously clear from Fig. 5 that *P. corylophilum* colony was dark green at the center of the colony, however the edges of the colony were white to greenish white. The mycelium of *P. corylophilum* had high branched networks of multinucleated cells located on a septum lacking hyphae. Conidiophores are at the end of each branch accompanied by green spherical constricted units called conidiospores as described by McMullin et al. (2014).

Conclusion

Thirty five isolates of fungi were isolated from eighty samples of different types of dairy products and morphologically identified. The results revealed that all isolates fell into five fungi species classified into (four species of *Aspergillus terreus*, nine of *Aspergillus niger*, nine of *Aspergillus flavus*, eight of *Aspergillus parasiticus*, and five of *Penicillium corylophilum*).

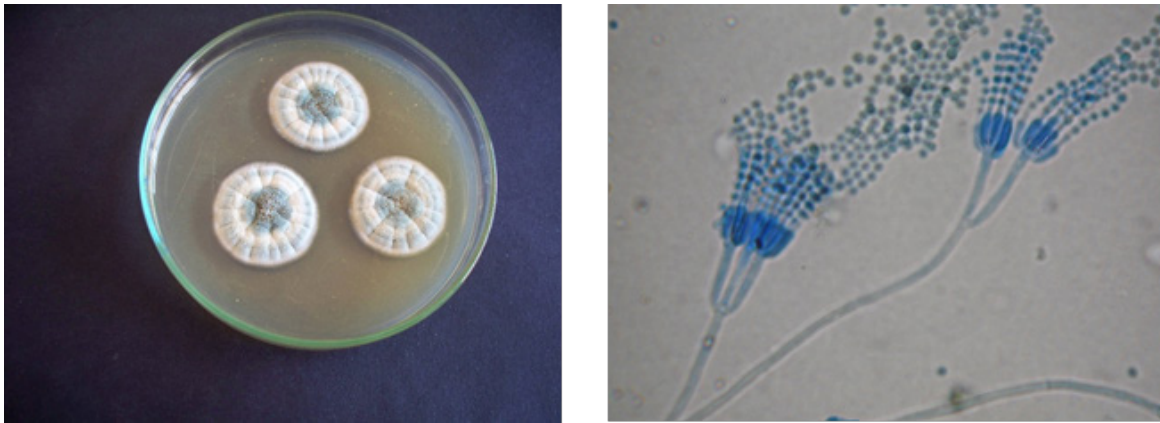


Fig. 5. *Penicillium corylophilum* colony on CYA medium and its microscopic image.

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الخصائص المورفولوجية لبعض أنواع الفطريات المعزولة من بعض منتجات الألبان في محافظة الفيوم

تم جمع ٨٠ عينة من منتجات الألبان المختلفة من الأسواق المختلفة في محافظة الفيوم. ولقد أُخبرت العينات من حيث وجود الفطريات من عدمه. حيث تم عزل ٢٥ عينة من الفطريات المختلفة ثم عرفت مورفولوجيا. حيث إعتد التعريف المورفولوجي للفطريات المعزولة أساسا على (اللون والتركيب) والمظهر وشكل metulae ووجود أو غياب الـ conidiophores تحت الميكروسكوب والذي يشمل الشكل وتفرع الـ S abouraud D extrose Agar وأخيرا تركيب الكونديا. ولقد عزلت الفطريات على بيئة phialides للتعريف المورفولوجي لمستعمرات (CYA) C zapek Yeast E x tract A gar (CYA). وأستخدمت بيئة SDA L actophenol cotton blue الفطريات المعزولة. بينما استخدمت صبغة اللاكتوفينول قطن الزرقاء لصبغ الشرائح الميكروسكوبية للتجهيز للفحص الميكروسكوبي. ولقد تم أخذ الصور الميكروسكوبية بكاميرا رقمية من نوع كانون 6G على قوة تكبير ٠٠٠١ مرة. وأخيرا أشارت النتائج إلى أن جميع السلالات التي تم عزلها (٢٥ سلالة) وجد انها تنتمي الى خمسة أنواع من الفطريات والتي قسمت إلى (أربعة *Aspergillus* تابعة لـ *Aspergillus niger* تسعة تابعة لـ *Aspergillus terreus* تابعة لـ *Penicillium corylophilum* وخمسة تابعة لـ *Aspergillus parasiticus* ثمانية تابعة لـ *Aspergillus flavus*).