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Isolation and Identification of Egyptian Ras Cheese (Romy) Contaminating Fungi during Ripening Period

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Abstract The fungal counts on Ras cheese samples obtained from ripening rooms of different factories from were determined. The lowest total fungal count was in Akel's factory samples, but El-Eman's factory was the highest count being 0.8×10^5 and 1.6×10^5 colony forming unit/gram (cfu/g), respectively. A total of 66 fungal isolates were examined in this study. The classification position of obtained fungal isolates were classified in three families (Endomycetaceae, Mucoraceae and Trichocomaceae), 6 genus and 13 species as following *Geotrichum candidum*, *Aspergillus ochraceus*, *A. alliaceus*, *A. oryzae*, *A. niger*, *A. nidulans*, *Emericella nidulans*, *A. flavus*, *A. glaucus*, *A. flavipes*, *Penicillium* sp., *Mucor* sp. and *Rhizopus stolonifer*. Most of fungal strains were found in El-Ashmawy's factory and Abdo Gohar's factory being 7 strains, but Akel's factory and El-Eman's factory were lower being 5 species and the last were El-Safa's factory and El-Faiomy's factory being 4 strains belonging to the genus *Aspergillus* being *A. ochraceus*; *A. oryzae*; *A. niger* and *A. glaucus*. *A. oryzae* was observed in all factories except Abdo Gohar and El-Eman's factories being 39.39% while the other strains were attributed according to their percentages. *A. glaucus* was in the second order being16.67% that presented also in all factories except El-Ashmawy's factory. *A. ochraceus* was in the third order being 15.15% that observed in all factories. *A. niger* which the fourth order being 6.66% that presented in El-Ashmawy, Abdo Gohar and El-Faiomy factories. Moreover, the distribution of other fungi were only in two factories, the percentage of *Geotrichum candidum* was 4.55% that presented in El-Ashmawy and Abdo Gohar factories. But, *Aspergillus flavus* was 4.55% and presented in Abdo Gohar and Akel factories. *Penicillium* sp. was 3.03% in El-Ashmawy and El-Eman factories. Finaly, *Rhizopus* sp. was 3.03% in El-Safa and El-Eman factories.

Keywords Fungal isolation, Fungal identification, Egyptian Ras cheese (Romy), Ripening rooms

1. Introduction

Ras cheese (Romy) is the main traditional hard cheese in Egypt, it is manufactured in a high proportion under artisan conditions from raw cow's or mixture of cow's and buffalo's milk without using starter cultures and marketing when it has a queried sharp flavor closed to kefalotyic cheese after 3 to 6 months [1, 2].

Cheese ripening is a complex and dynamic biochemical process that includes protein breakdown, fat hydrolysis and lactose metabolism. These processes are catalyzed by agents such as residual coagulant, indigenous milk enzymes, starter or nonstarter microflora and secondary organisms. The secondary organisms include moulds and presence of moulds on the surface of mould-ripened cheese gives them a different appearance and flavor from other cheeses. The moulds have more complex enzymatic systems than bacteria and their enzymes contribute in cheese maturing of the cheese, i.e., to proteolysis and lipolysis which are more extensive in these cheeses [3].

Fungi are significant spoilage microorganisms of foodstuffs during the storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Fungal growth on cheese is a common problem for the cheese manufacture during ripening and curing as well as for the retailer and consumer during refrigeration storage. Species of *Penicillium* and *Aspergillus* are common contaminants of cheese [4]. By the searching in the medical references, it was observed that, most of this fungi had the ability to human and animal pathogenicity or produced toxins [5-12].

The growth of toxigenic fungi during ripening of Ras cheese must be considered as a problem of safety for human consumption. During the ripening of Ras cheese, non-toxigenic strains of fungi should be avoid, moreover, fungi growth on the cheese surface causes economic losses and quality problems. Our isolated fungi will tested for their mycotoxin production, selected and will used as an inoculum in an attempt to control Ras cheese contamination during storage period.

This work aims to isolation and identification of fungi

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growing on the Egyptian Ras cheese during ripening period.

2. Materials and Methods

2.1. Cheese Samples

Samples of Egyptian Ras cheese were collected from ripening rooms of different factories (Fa) during (June of 2013), namely El-Ashmawy (AL-Sharqia governorate) (Fa1), Abdo Gohar (Kafr EL Sheikh governorate) (Fa2), El-Safa (Dakahlia governorate) (Fa3), El-Faiomy (Damittae governorate) (Fa4), Akel (Damittae governorate) (Fa5) and El-Eman (Kafr EL Sheikh governorate) (Fa6).

2.2. Cultivation Media

Potato dextrose agar (PDA) medium [13]; Rose Bengal yeast extract sucrose agar (RYS) medium [14] and Czapek yeast extract agar with 20% sucrose (CY20S) medium [14] were used for isolation and identification of fungi from examined Ras chees.

2.3. Fungal Count and Isolation

Scraping of Ras cheese wheel surface was done to get the contaminate fungal load then mixed well and 1.0 gm was taken for isolation. One gram of the above sample was suspended in 9.0 ml sterilized tap water then serial dilutions from 10-1 to 10-5 were done using a vortex (VM-300 power: 220 VAC, 50Hz, 0.16A/Made in Taiwan-Associated with Cannic, inc U.S.A.) to homogenate the obtained solution. One ml of each last three dilutions (10-3, 10-4 and 10-5) was put into petri dish then melted PDA medium was poured and mixed well then left to solidify. These petri dishes were then incubated in a digital incubator (Switc, MPM Instruments s.r.l., Bernareggio/Made in Italy) at 25°C for 5 days. Then single colonies of fungi developed on PDA medium were counted and picked-up [15]. Colonies were transferred to PDA plates for sub culturing to obtain pure fungal isolates [16]. The fungal isolates were maintained on PDA slants at 5°C till use. Before use, the fungal isolates were subcultured on new slants of PDA and incubated at 25°C for 5 days.

2.4. Identification of Obtained Fungal isolates

Identification was based on the visual observation of fungal isolates grown on PDA plates. Fungal isolates were identified by morphological characteristics of colonies in different cultivation media such as PDA, RYS and CY20S. In addition, the vegetative and reproduction strictness observed using a light microscope (Olympus CX31 Binocular Halogen Microscope made in Japan) with a magnification power 400x, were also considered. The following taxonomic keys were used [4, 11, 17-24].

3. Results and Discussion

3.1. Fungal Counts, Isolation and Identification

Fungal counts of Ras cheese samples are presented in Table 1. Akel's factory (Fa5) samples had the lowest total fungal count but El-Eman's factory (Fa6) had the highest count being 0.8×10^5 and 1.6×10^5 (cfu/g), respectively. This result is in line with those Florez and Mayo [25] who studied Cabrales cheese the most famous of the traditional, Spanish, blue veined cheeses and they found that, Cabrales cheese had been subject microbial characterization. Colonies of all morphologies from this culturing study were chosen (48 yeasts and 35 moulds). Mould and yeasts isolates were classified by morphotypic and biochemical criteria. Also, Hayaloglu and Kirbag, [3] studied the fungal growing on the surface of mould-ripened cheeses variety produced in the middle and eastern region of Turkey and they found that, a total of 24 different species of mould were isolated and identified in Kuflu cheese samples. These species made up 36.7% of the total number of moulds. The genus most frequently isolated was *Penicillium* spp. Which represented 70.25% (111 isolates of *Penicillium* spp.) of total isolates. Other genera isolated were *Alternaria*, *Acremonium*, *Aspergillus*, *Cladosporium*, *Geotrichum*, *Mucor*, *Rhizopus* and *Trichoderma*. Similar results was obtained by Santi *et al*., [26] who, isolated and identified filamentous fungi from traditional Italian cheese (Fossa cheese) and its ripening environment. After ageing for 60 days at a dairy, it was ripened for an additional three months in a pit. In the fully ripened cheese, moulds ranged from 3 to 3.4 log cfu/g. Pit environmental fungi ranged from 530 to 750 cfu/m^3 (air) and from 130 to 340 cfu/cm^2 (surfaces).

Table 1. Fungal counts of Ras cheese samples

Sample examined	Fungal count (cfu x 10^5 /g Ras cheese)
El-Ashmawy (Fa1)	1.2
Abdo Gohar (Fa2)	0.9
El-Safa (Fa3)	1.3
El-Faiomy (Fa4)	0.9
Akel (Fa5)	0.8
El-Eman (Fa6)	1.6

A total of 66 fungal isolates obtained were examined in this study. Thirty different isolates were isolated from all samples. Among all samples, El-Eman's factory (Fa6) was the highest count of fungal isolate being 17 isolates, but Akel's factory (Fa5) was the lowest count being 8 fungal isolates. Fungal isolate number 9 was observed 26 times and it was observed in the most samples, but fungal isolate numbers 3, 6, 7, 11 and 13 were observed only one time.

3.2. Characterization and Identification of Fungal Isolates

The cultural characteristics of the fungal isolates showed that, colonies were white, dry, powdery to cottony colonies on PDA medium (Fig. 1). When disturbed on the surface, the colony becomes yeast-like or slimy. The microscopic

features showed that, arthroconidia and coarse true hyphae were observed. Blastoconidia, conidiophores and pseudohyphae were absent. They are either rectangular in shape or rounded at the ends resembling the barrel shape. From these characteristics, isolate No. 1, 2 and 35 were identified as *Geotrichum candidum* following the protocol of Buchta and Otcenasek [19]. Similar result was obtained by Hayaloglu and Kirbag, [3] who studied the semihard texture, rectangular shape Kuflu cheese which was a mould-ripened variety produced in the middle and eastern region of Turkey. This cheese is made from non-pasteurized, skimmed (or partially-skimmed) sheep's or goat's milk or their mixture. *Penicillium* sp, and *Geotrichum candidum* were the most frequently isolates. Also, a total of 24 fungi were isolated from Kuflu cheese being *Aspergillus fumigatus*, *A. flavus*, *Rhizopus nigricans*, *Acremonium alternatum*, *Alternaria alternate*, *A. niger*, *Cladosporium cladosporioides*, *C. herbarium*, *Mucor himealis*, *Rhizopus nigricans* and *Trichoderma harzianum*. Moreover, Cathrine and Skaar, [27]; Kure *et al*., [28] and Florez *et al*., [29] isolated and identified *Geotrichum candidum* from Norwegian semi-hard cheeses, Jarlsberg cheese and Spanish blue-veined Cabrales cheese.

Figure 1. *Geotrichum candidum* on RYS medium

The colonies of isolates Nos. 3, 4, 14, 28, 31, 38, 49, 57, 65 and 66 grew rapidly on PDA medium (Fig. 2) while the conidial heads are typically arranged in zones. The color of the colony is yellow. This isolate formed pinkish to purple color on PDA medium, irregular, pebble like, the conidiophores appear as powdery mass. Microscopically, the appearances of these conidiophores are granular with pale yellow-brown walls that attach abruptly to a "globose to subglobose vesicle" (Fig. 3). From these characteristics, these isolates were identified as *Aspergillus ochraceus* following Chung and Bennett [21].

Few studies on the presence of *Aspergillus ochraceus* in cheese have been published [30]. Particularly worthy of mention are those by Spanish [31], Turkish [32] and Italian researchers [33]. In Egypt, Abdel-All *et al*., [34] isolated and identified twenty five of different fungi from different Gouda cheese samples and they found that, *A. parasiticus*, *A. flavus*, *A. niger*, *A. versicolor*, *A. terrus*, *A. ochraceus*, *A. candidus*, *A. nidulans*, *P. requforti* and *P. chresogenum* were the most isolated fungi. Also, Pattono *et al*., [30] reported that, Ochratoxin A is produced by different species of the genus *Aspergillus* such as *A. ochraceus*, *A. melleus*, *A. sulphureus*, *A. niger*, *A. lanosus*, *A. alliaceus*, *A. carbonarius* and *A. awamori* and different species of the genus *Penicillium* such as *P. verrucosum*, *P. chrysogenum* and *P. nordicum*.

Figure 2. *Aspergillus ochraceus* on PDA medium

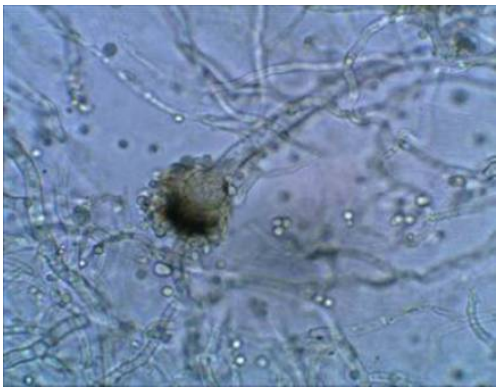

Figure 3. *Aspergillus ochraceus* under light microscope (400x)

The characteristics of isolate No. 30 showed to be green, on PDA medium (Fig. 4). The isolate grew predominantly as sterile hyphae interspersed with large ellipsoidal to cylindrical dark brown ascomata produced directly from the vegetative hyphae. Conidial heads indicative of the genus *Aspergillus*. Conidiophores were smooth with globose vesicles that gave rise to radiating, biseriate conidial heads producing smooth-walled, globose conidia. From these characteristics and following the protocol of Balajee *et al*., [11] isolate No. 30 was identified as *Aspergillus alliaceus* (recently *Petromyces alliaceus*).

Figure 4. *Aspergillus alliaceus* on RYS medium

They found that, with the combined phenotypic and genotypic evidence, the unknown fungal isolate was identified as *P. alliaceus*. The teleomorphic species *P. alliaceus* is one of only three members assigned to the genus *Petromyces*. Although *Petromyces* spp. have been isolated from surfaces of plants and soil, the *P. alliaceus* is rarely recovered from an invasive fungal infection and has been previously isolated from a case of chronic otorrhea (drainage from the auditory canal). *Petromyces alliaceus* produces a variety of secondary metabolites, including ochratoxin A and B. Although the anamorphous of the teleomorphic genus *Petromyces* was originally assigned to the *Aspergillus* section Circumdati, recent chemotaxonomic and genotypic evidence places this genus in *Aspergillus* section Flavi, whose members often produce sclerotia [11]. The fungal contamination of Gouda cheese was studied by Berlkten and Kivanc [35] and they isolated and identified 21 isolates as *Aspergillus alliaceus*, *Geotrichum candidum* and *Penicillium* sp.

Characterization of the isolates showed that, colonies were yellow green on Rose Bengal medium (Fig. 5), conidiophores colorless, long, coarsely roughened. conidial heads typically radiate, conidia globose to subglobose. From these characteristics following the schemes of Chung and Bennett [21], isolate Nos. 13, 15, 16, 17, 21, 22, 29, 33, 34, 37, 39, 40, 43, 45, 50, 52, 54, 58, 59, 60, 61, 62, 63, 67, 69 and 70 were identified as *Aspergillus oryzae*.

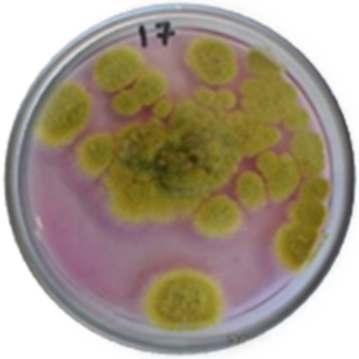

Figure 5. *Aspergillus oryzae* on RYS medium

The fungal contamination of cheese at the stage of consumption in Saudi Arabia (including Egyptian cheese) was studied by Nasser, [36] who found that, 13 species of fungi were isolated from 12 cheese samples. The total fungal counts in cheese samples were relatively low and ranged from 95 and 125 cfu/g. Members of *Penicillium* and *Aspergillus* (*A. flavus*, *A. niger* and *A. oryzae*) were the most prevalent fungi on cheese samples. Ando *et al*., [37] isolated *A. oryzae* from Iranian commercial cheese (Caspian cheese). Also, *A. oryzae* was isolated from spoiled cheese by Sharma et al., [38] and used it for producing alkaline protease.

Mycelia of colonies of isolates Nos. 7, 27, 28 and 46 on PDA medium were white, conidial heads dark brown, greenish black (Fig. 6), brownish black to black reverse colorless, conidial heads globose, radiate or splitting into several irregular or well-defined columns of conidial chains, conidiophores hyaline to brown and smooth-walled (Fig. 7). Vesicles globose to subglobose hyaline to dark brown. From these characteristics, these isolates were identified as *Aspergillus niger* according to the protocol of Chung and Bennett [21]. Many authors isolated *A. niger* from cheese such as; Hassan and El-Deeb, [39] (Ras cheese); El-Deeb *et al*., [40] (Ras cheese); Nasser, [36] (white cheese); Hayaloglu and Kirbag, [3] (Kuflu cheese); Abdel-All *et al*., [34] (Ras cheese); Anwar and Sabah [41] (Qastqawan cheese) and Santi *et al*., [26] (Fossa cheese).

Figure 6. *Aspergillus niger* on PDA medium

Figure 7. *Aspergillus niger* under light microscope (400x)

Some isolates showed pale green or dark yellow green colonies PDA on (Fig. 8), Conidiophores rarely exceed long, colourless, smooth, columnar, conidial heads looscly columanar vesicles very small, conidia globose or subglobose, smooth, ascospores present (Fig. 9). From these characteristics, isolate No. 26 was identified as *Aspergillus nidulans* [21].

Similar results were obtained by Barrios *et al*., [42] who reported that, contamination by the genus *Aspergillus* was studied in 52 samples of commercial cheeses made with different types of milk (cow's milk, ewe's milk, goat's milk and mixture of them) produced in southern Spain. The frequency of appearance of various species of the genus *Aspergillus* such as *A. glaucus, A. niger, A. nidulans, A. sulphureus, A. terreus, and A. flavus,* in the different types of cheese was determined. Also, Abdel-All *et al*., [34]; Santi *et al*., [26] and Ando *et al*., [37] isolated *A. nidulans* from Gouda, Fossa cheese and Caspian cheese, respectively.

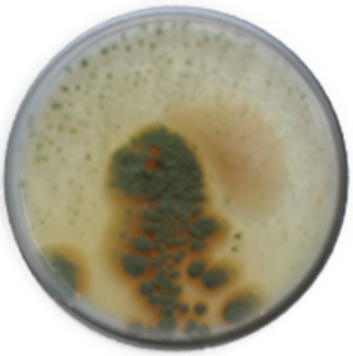

Figure 8. *Aspergillus nidulans* on PDA medium

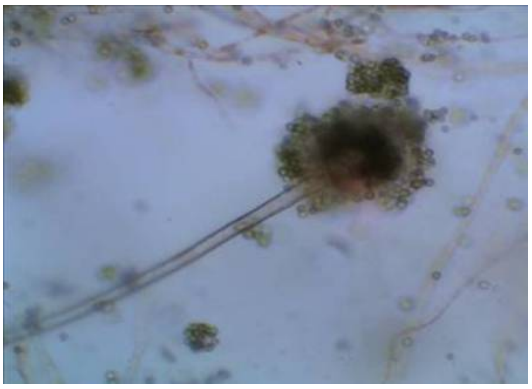

Figure 9. *Aspergillus nidulans* under light microscope (400x)

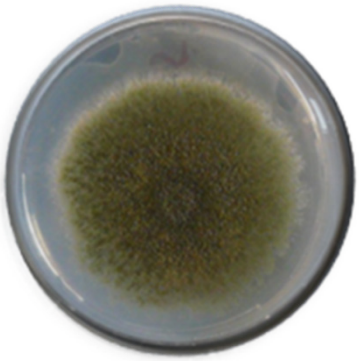

Figure 10. *Emericella nidulans* on PDA medium

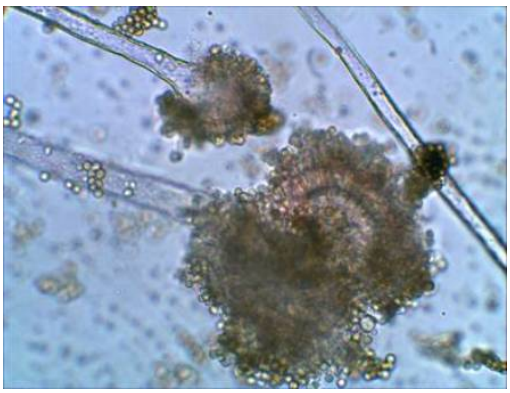

Figure 11. *Emericella nidulans* under light microscope (400x)

Cleistothecia and conidial structures of isolate No. 8 duplicating those of nidulans, ascosp orange red on PDA medium (Fig. 10), lenticular, convex wall smooth, equatorial ridges absent (Fig. 11), but ascospres showing the usual bivalve construction. From these characteristics, isolate No. 8 was identified as *Emericella nidulans* [21].

Isolates Nos. 18, 23 and 24 showed conidial heads pale to intense yellow green when young, colonies not shifting to brown in age on PDA medium (Fig. 12); conidia definitely echinulate predominane; conidial heads radiate or very loosely columnar, colonies shifting to brownish in age; conidia smooth to roughened; conidiophores arising primarily from the substrate (Fig. 13). From these characteristics, these isolates were identified as *Aspergillus flavus* [21]. Many authors isolated *A. flavus* from cheese such as; Hassan and El-Deeb, [39] (Ras cheese); El-Deeb *et al*., [40] (Ras cheese); Nasser, [36] (white cheese); Hayaloglu and Kirbag, [3]; (Kuflu cheese); Abdel-All *et al*., [34] (Ras cheese); Gandomi *et al*., [4] (white cheese); Anwar and Sabah [41] (Qastqawan cheese) and Santi *et al*., [26] (Fossa cheese).

Figure 12. *Aspergillus flavus* on PDA medium

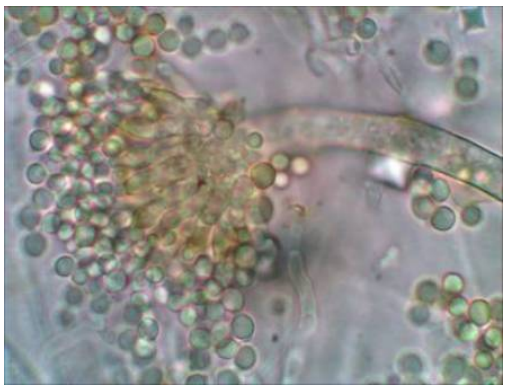

Figure 13. *Aspergillus flavus* under light microscope (400x)

Colonies of isolates Nos. 5, 6, 9, 10, 15, 19, 41, 44, 47, 48 and 53 on PDA medium were grayish turquoise to deep green (Fig. 14). Reverse was pale yellow to pale brown hyphae are septate and hyaline with a cleistothecia. Conidial heads were radiate to loosely columnar. Conidiophores were smooth walled and uncolored to pale brown. Vesicles were globose to subglobose and uniseriate. Conidia were globose to subglobose (Fig. 15). From these characteristics, these isolates were identified as *Aspergillus glaucus* [20]. Similar results were obtained by Barrios *et al*., [42] who isolated *A.*

glaucus from commercial cheeses produced in southern Spain. Also, Santi *et al*., [26] isolated and identified *Aspergillus glaucus*, from traditional Italian cheese (Fossa cheese).

Figure 14. *Aspergillus glaucus* on RYS medium

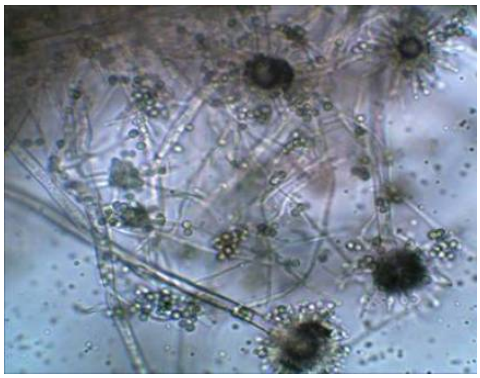

Figure 15. *Aspergillus glaucus* under light microscope (400x)

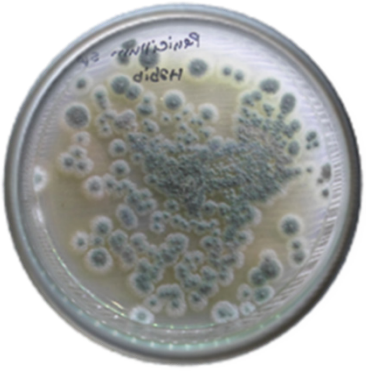

Figure 16. *Penicillium* sp. on PDA medium

Figure 17. *Penicillium* sp. under light microscope (400x)

The colonies of isolate Nos. 11 and 68 were rapid grown, flat, filamentous, and velvety, woolly, or cottony in texture on PDA medium (Fig. 16). The colonies were initially white and become blue green, gray green, olive gray, yellow or pinkish in time. visualized as globose to elongated sausage-shaped cells that multiply by fission (Fig. 17). From these characteristics, isolates No. 11 and 68 was identified and designated as *Penicillium* sp. [24].

Many authors isolated *Penicillium* from cheese such as; Hassan and El-Deeb [39] (Ras cheese); El-Deeb *et al*., [40] (Ras cheese); Cathrine and Skaar, [27] (Norwegian semi-hard cheeses); Kure *et al*., [28] (Jarlsberg cheeses); Nasser, [36] (white cheese); Florez *et al*., [29]; Hayaloglu and Kirbag, [3] (Kuflu cheese); Abdel-All *et al*., [34] (Ras cheese); Gandomi *et al*., [4] (white cheese), Anwar and Sabah [41] (Qastqawan cheese) and Santi *et al*., [26] (Fossa cheese). *Penicillium roqueforti* and *P. commune* were also isolated from spoilage Cheddar cheese by Taniwaki *et al*., [43]. Moreover, Ando *et al*., [37] isolated *P. crysogenum* and *P. citrinum* from Iranian commercial cheese (Caspian cheese).

Colonies of isolate No. 12 grow rapidly at 25°C and quickly covered the surface of the medium. Its fluffy appearance with a height of several cm resembles cotton candy. From the front, the color is white initially and becomes grayish brown in time on Rose Bengal medium (Fig. 18). From the reverse, it was white. Nonseptate or sparsely septate, broad hyphae, sporangiophores, sporangia, and spores were visualized. Intercalary or terminal arthrospores (oidia) located through or at the end of the hyphae and few chlamydospores maybe also produced by some species. Apophysis, rhizoid and stolon were absent. Sporangiophores are short, erect, taper towards their apices and may form short sympodial branches. Columella were hyaline or dematiaceous and were hardly visible if the sporangium has not been ruptured. Smaller sporangia may lack columella. Sporangia were round, gray to black in color, and filled with sporangiospores. From these characteristics, isolate No. 12 was identified as *Mucor* sp. [23].

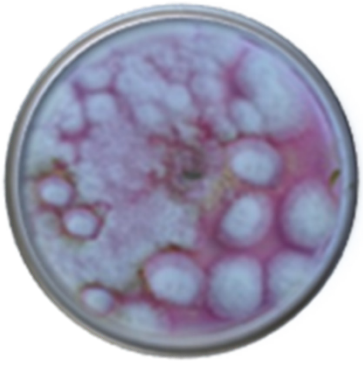

Figure 18. *Mucor* sp. on RYS medium

The growth of *Mucor plumbeus* and mycotoxin production on cheese under modified atmospheres was studied by Taniwaki *et al*., [43]. Also, *Mucor racemosus* and *Mucor* sp. were isolated and identified from Qashqawan

Cheese and traditional Italian cheese (Fossa cheese) by Anwar and Sabah [41] and Santi *et al*., [26], respectively. Ando *et al*., [37] isolated *Mucor hiemalis*, *M. javanicus* and *M. roxianus* from Iranian commercial cheese (Caspian cheese). Also, Cheong *et al*., [44] reported that, Moulds are the most common cheese spoilage organisms which can lead to economic loss as well as raising public health such as *Penicillium solitum*, *Aspergillus versicolor* and *Cladosporium herbarum*, *Mucor circinelloides* and *Geotrichum candidum*. On the other hand, Lynch *et al.*, [45] published that, the most frequently isolated fungi from cheese were *Alternaria*, *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, *Mucor*, *Penicillium* and *Phoma*.

Colonies of isolate Nos. 42 and 64 grew very rapidly, fill the Petri dish, and mature in 4 days. The texture is typically cotton-candy like on PDA medium (Fig. 19). From the above data, the color of the colony was white initially and turns grey to yellowish brown in time. The reverse was white to pale. Nonseptate or sparsely septate broad hyphae, sporangiophores, rhizoids (root-like hyphae), sporangia, and sporangiospores were visualized. Sporangiophores were brown in color and usually unbranched (Fig. 20). They can be solitary or form clusters. Rhizoids were located at the point where the stolons and sporangiophores meet. Sporangiospores are unicellular, round to ovoid in shape, hyaline to brown in color, and smooth or striated in texture. From these characteristics, isolates Nos. 42 and 64 were identified as *Rhizopus stolonifer* [8]. Also, *Rhizopus* sp. was isolated and identified by Santi *et al*., [26] from traditional Italian cheese (Fossa cheese). In addition, Ando *et al*., [37] isolated *Rhizopus retlexus*, *R. stolonifer* and *R. javanicus* from Iranian commercial cheese (Caspian cheese).

Figure 19. *Rhizopus stolonifer* on PDA medium

Figure 20. *Rhizopus stolonifer* under light microscope (400x)

Conidiophores of isolate No. 56 were smooth, in yellow to light brown shades or colourless on PDA medium (Fig. 21). Conidial heads radiate when young, becoming broadly to irregularly columnar in age; persistently with or changing to avellaneous, pllebuff or greyish buff. Vesicles subglobose to ovate or elongate. Metulae present, covering the entire surface of the vesicle, or only the upper part. Conidia globosse to subglobose, colourless, smooth. Ascomata known in tow species; ascospores hyaline to pale yellow. Conidiophores definitely pigmented in yellow to light brown shades, conidial heads were white to very pale buff. From these characteristics, isolate No. 56 was identified as *Aspergillus flavipes* [21]. *Alternaria*, *A. flavus*, *A. niger*, *A. glaucus*, *A. flavipes*, *A. terreus*, *Cladosporium* sp., *Fusarium solani*, *Mucor* sp., *Penicillium* sp., *Phoma* sp. and *Rhizopus* sp. were isolated and identified by Santi *et al*., [26] from traditional Italian cheese (Fossa cheese).

Figure 21. *Aspergillus flavipes* on PDA medium

3.3. The Classification Position of Fungal Isolates

Table 2 show that, the classification position of obtained fungal isolates were classified in three families, 6 genus and 13 species as following; one species was belonging to family Endomycetaceae being *Geotrichum candidum*. Two species were belonging to family Mucoraceae being *Mucor* sp. and *Rhizopus* sp. The other eleven fungi were belonging to family Trichocomaceae being *A. ochraceus; A. alliaceus; A. oryzae; A. niger; A. flavus; A. glaucus; A. flavipes; A. nidulans; Emericella nidulans and Penicillium* sp. [21].

Most of fungal strains were found in El-Ashmawy and Abdo Gohar being 7 species, but Akel and El-Eman Factories were the second in fungal strains being 5 strains and the third were El-Safa and El-Faiomy being 4 strains. Obtained results is in agreement with those obtained by Florez and Mayo [25] who identified fungal isolates during manufacturing and ripening of Cabrales cheese. They found, 12 isolates of *Penicillium roqueforti*, 11 isolates of *Penicillium*, 10 isolates of *G. candidum*, one isolate *Acremonium charticola* and one unidentified isolate. *G. candidum* was frequently isolated from one of four cheese factories in Norway. A few isolates of other genera including *Acremonium*, *Alternaria*, *Cladosporium*, *Mucor* and *Trichoderma* spp. were also found on Kuflu cheese.

Table 2. The Classification of fungal isolates

Family	Genus	Species
Endomycetaceae	*Geotrichum*	*candidum*
Trichocomaceae	*Aspergillus*	*ochraceus*
		alliaceus
		oryzae
		niger
		flavus
		glaucus
		flavipes
		nidulans
	Emericella	*nidulans*
	Penicillium	sp.
Mucoraceae	*Mucor*	sp.
	Rhizopus	*stolonifer*

3.4. Incident of Fungal Strains on the Ras Cheese

Table 3. shows, the presence of fungal strains of Ras cheese samples obtained from six different factories. *Aspergillus oryzae* was observed in all factories except Abdo Gohar and El-Eman 's factories being 39.39% while the other strains were attributed according to their percentages. *Aspergillus glaucus* was in the second order being16.67% that presented also in all factories except El-Ashmawy's factory. *Aspergillus ochraceus* was in the third order being 15.15% that observed in all factories. *Aspergillus niger* which the fourth order being 6.66% that presented in El-Ashmawy, Abdo Gohar and El-Faiomy factories.

Table 3. The distribution of fungal species on the Egyptian Ras cheese factories

Fungal species	Factory names						∑	%
	El-Ashmawy	Akel	Abdo Gohar	El-Safa	El-Faiomy	El-Eman	6	
Geotrichum candidum	√	-	√	-	-	-	2	4.55
Aspergillus ochraceus	√	√	√	√	√	√	6	15.15
Aspergillus alliaceus	-	-	√	-	-	-	1	1.52
Aspergillus oryzae	√	√	√	√	√	√	6	39.39
Aspergillus niger	√	-	√	-	√	-	3	6.66
Aspergillus nidulans	-	-	√	-	-	-	1	1.52
Emericella nidulans	√	-	-	-	-	-	1	1.52
Aspergillus flavus	-	√	√	-	-	-	2	4.55
Aspergillus glaucus	-	√	√	√	√	√	5	16.67
Penicillium sp.	√	-	-	-	-	√	2	3.03
Mucor sp.	√	-	-	-	-	-	1	1.52
Rhizopus stolonifer	-	-	-	√	-	√	2	3.03
Aspergillus flavipes	-	-	-	-	-	√	1	1.52
∑	7	5	7	4	4	5	-	100

The distribution of the following fungi were only in two factories, the percentage of *Geotrichum candidum* was 4.55% that presented in El-Ashmawy and Abdo Gohar factories. The percentage of *Aspergillus flavus* was 4.55% that presented in Abdo Gohar and Akel factories. The percentage of *Penicillium* sp. was 3.03% and was presented in El-Ashmawy and El-Eman factories. The percentage of *Rhizopus* sp. was 3.03% and was presented in El-Safa and El-Eman factories. Obtained results are in agreement with those obtained by Florez and Mayo [25] who identified fungal isolates during manufacture and ripening of Cabrales cheese. They found that, 12 isolates of *Penicillium roqueforti*, 11 isolates of *Penicillium*, 10 isolates of *Geotrichum candidum*, one isolate *Acremonium charticola* and one unidentified isolate. Finally, *Aspergillus alliaceus* and *Aspergillus nidulans* presented in only one factory (Abdo Gohar's factory), in a percentage of 1.52%, for each. Also, *Emericella nidulans* and *Mucor sp.* were 1.52% for each one found in El-Ashmawy factory. While, *Aspergillus flavipes* was found in El-Eman Factory only.

4. Conclusions

It can be concluded that, most of fungal strains were found in El-Ashmawy and Abdo Gohar being 7 strains, but Akel and El-Eman factories were the second in fungal species being 5 species and the third were El-Safa and El-Faiomy being 4 strains. Moreover, Uncontrolled fungi growth on the cheese surface must be considered as a problem and provides no guarantee of product safety for human consumption. For the ripening of Ras cheese, non-toxigenic strains of fungi should be selected

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