Molecular Identification of Yeasts Associated with Traditional Egyptian Dairy Products

W.M. El-Sharoud, C. Belloch, D. Peris, and A. Querol

ABSTRACT: This study aimed to examine the diversity and ecology of yeasts associated with traditional Egyptian dairy products employing molecular techniques in yeast identification. A total of 120 samples of fresh and stored Domiati cheese, kariesh cheese, and "Matared" cream were collected from local markets and examined. Forty yeast isolates were cultured from these samples and identified using the restriction-fragment length polymorphism (RFLPs) of 5.8S-ITS rDNA region and sequencing of the domains D1 and D2 of the 26S rRNA gene. Yeasts were identified as *Issatchenkia orientalis* (13 isolates), *Candida albicans* (4 isolates), *Clavispora lusitaniae* (*Candida lusitaniae*) (9 isolates), *Kodamaea ohmeri* (*Pichia ohmeri*) (1 isolate), *Kluyveromyces marxianus* (6 isolates), and *Candida catenulata* (7 isolates). With the exception of *C. lusitaniae*, the D1/D2 26S rRNA gene sequences were 100% identical for the yeast isolates within the same species. Phylogenetic reconstruction of *C. lusitaniae* isolates grouped them into 3 distinguished clusters. Kariesh cheese was found to be the most diverse in its yeast floras and contained the highest total yeast count compared with other examined dairy products. This was linked to the acidic pH and lower salt content of this cheese, which favor the growth and survival of yeasts in foodstuffs. Stored Domiati cheese also contained diverse yeast species involving isolates of the pathogenic yeast *C. albicans*. This raises the possibility of dairy products being vehicles of transmission of pathogenic yeasts.

Keywords: cheese, dairy products, molecular identification, yeasts

Introduction

Yeasts play diverse roles in affecting the quality and safety of food products. They have been traditionally used for the preparation of bread, beer, and wine. Some yeasts have been also shown to contribute to the development of desirable flavor during cheese ripening, and are thus considered for use as starter cultures (Loretan and others 1998). Because of their antagonistic action toward fungi, certain yeasts have been developed as biocontrol agents of food spoilage fungi, and others are being considered as novel probiotic organisms (Fleet 2007). However, several yeasts metabolize organic acids in fermented foods causing an increase in the pH and thus allowing the growth of spoilage and pathogenic bacteria (Viljoen 2006). Some yeasts may further pose threats to food safety given their association with opportunistic infections and other adverse conditions in humans (Fleet 2007).

There is a growing interest in the biodiversity and ecology of yeasts associated with different foods (Fleet 2007). This is due to the realization that yeasts can interact with themselves and other microbial species in different ecosystems and that the outcome of these interactions may affect the role(s) that yeasts have in foods. It is therefore relevant to identify yeasts associated with different foods and establish relationships between the characteristics of examined foods and the diversity of their yeast floras. Traditionally, physiological and morphological examinations have been employed for the identification of yeasts in foods (Barnett and others 2000). However, it is becoming evident that these tests are not adequate for the delineation and identification of yeast species (Prillinger and others 1999). They show high variability and cannot be relied on for proper identification of yeasts (Prillinger and others 1999; Barnett and others 2000). The application of molecular methodologies to the identification of yeasts has provided a useful tool for studying the biodiversity of yeasts in foods (Prillinger and others 1999; Fleet 2007). These methods involve the analysis of the coenzyme Q system and the monosaccharide pattern of cell walls, random amplification of polymorphic DNA (RAPD), microsatellite analysis, restriction fragment length polymorphism (RFLP) of transcribed and spacer sequences of ribosomal DNA, chromosome polymorphism determined by pulsed-field gel electrophoresis (PFGE), and sequencing of the 18S rRNA gene. Some of these methods have been successfully used for the identification of foodborne yeasts (Kurtzman and others 2003; Fernandez-Espinar and others 2006).

Traditional Egyptian dairy products are widely produced and consumed in the Mediterranean Sea area. These products include Domiati cheese (salted, enzyme-coagulated soft cheese), Kariesh cheese (slightly salted, acid-coagulated soft cheese), and Matared cream (naturally fermented cream prepared in earthenware pot called "Matared"). There are 2 varieties of Domiati cheese: fresh and stored, which differ from each other in the salting level of milk used for cheese making and storage time and conditions. Fresh Domiati cheese is less salted and stored for a few weeks under refrigeration, whereas stored Domiati cheese is highly salted and stored for a few months in brine solution or salted whey. The preparation of these traditional dairy products from raw milk with the application of less hygienic practices, compared with those adopted in large production facilities provide the possibility of their contamination with diverse microorganisms. While there have been several studies on the bacterial composition of these products (Roushdy and others 1998; El-Baradei and others 2007; El-Sharoud and Spano 2008; El-Sharoud 2009), little is known on the diversity of yeasts

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associated with these foods. In the present study, we used the restriction-fragment length polymorphism (RFLPs) of 5.8S-ITS rDNA region and sequencing of the domains D1 and D2 of the 26S rRNA gene for the identification of yeasts isolated from traditional Egyptian dairy products including fresh and stored Domiati cheese, Kariesh cheese, and "Matared" cream. Results showed different degrees of yeast diversity in examined products and that this was related to the moisture and salt contents and pH of these products.

Materials and Methods

Sampling of dairy products

A total of 120 samples of traditional Egyptian dairy products were aseptically collected from local markets in Mansoura, Kafer El-Sheikh, and Mahla, which are located in the Nile Delta region, Egypt. Examined samples included 30 specimens of each of fresh Domiati cheese (salted, enzyme-coagulated soft cheese, stored under refrigeration and consumed within a few weeks of preparation), stored Domiati cheese (highly salted, enzyme-coagulated soft cheese, stored in brine solution, or salted whey at room temperature for a few months before consumption), Kariesh cheese (slightly salted, acid-coagulated soft cheese, stored at room temperature, or under refrigeration to be consumed within a few days to 1 wk after preparation), and Matared cream (naturally fermented cream prepared in earthenware pot called "Matared" that is stored at room temperature or under refrigeration to be consumed within days to 1 wk after preparation). Samples were brought, cooled to the laboratory, and preserved under refrigeration until analysis within 3 h of sampling.

Enumeration and isolation of yeasts

A 10 g sample of each dairy product was homogenized in 90 mL quarter strength Ringers' solution (Oxoid, Basingstoke, U.K.) using a stomacher (Stomacher 400 Lab-blender, Seward Medical UAC House, London, U.K.) for 2 min at normal speed. Serial decimal dilutions of each homogenized sample were then prepared in quarter strength Ringers' solution and plated onto yeast extract glucose chloramphenicol agar (YGCA) (Merck, Darmstadt, Germany) (IDF 1990). Plates were incubated at 25 °C for 5 d and counted. Colonies showing differences in size, shape, or/and color were picked up and examined by microscopy for typical yeast cell morphology. Yeast colonies were purified on malt extract agar (Merck) and preserved on the same medium for identification.

PCR reactions

Yeast cells grown on GPYA (glucose 2%, peptone 0.5%, yeast extract 0.5%, and agar 2%) were collected with the tip of a toothpick from 48 h old colonies and directly used for PCR in a Progene Techne thermocycler (Cambridge, U.K.). PCR reactions consisted of an initial step at 95 °C for 5 min, 40 cycles of 94 °C for 40 s, 55 °C for 40 s, and 72 °C for 30 s, with a final extension of 10 min at 72 °C. Amplification of the 5.8S-ITS rDNA region was achieved with the primers its1 (5′-TCCGTAGGTGAACCTGCGG-3′) and its4 (5′-TCCTCCGCTTATTGATATGC-3′). Amplification of the D1/D2 domains of the 26S rRNA gene was done with the primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGT GTTTCAAGACGG-3′) (White and others 1990).

Restriction-fragment length polymorphism (RFLPs) of 5.8S-ITS rDNA

PCR products of the 5.8S-ITS region were digested without further purification with the restriction endonucleases *Cfo*I, *Hae*III, and *Hin*fI (Roche Diagnostics, Barcelona, Spain). PCR products and restriction fragments were separated on 1% and 3% agarose gels, respectively, in 0.5X TBE buffer. Band sizes were estimated by comparison against 100 and 50 bp DNA ladders (Invitrogen, Calif., U.S.A.). The RFLPs of the yeast isolates were compared with patterns of 300 yeast species in the Yeast-id database (http://www.yeast-id.com) and assigned to a known yeast species.

DNA sequencing

Amplified PCR D1/D2 26S rDNA products were purified (PerfectPrep Gel Cleanup, Eppendorf, Germany) and directly sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Calif., U.S.A.) in an Applied Biosystems (Model 310) automatic DNA sequencer. PCR primers NL-1 and NL-4 of the D1/D2 26S rRNA gene were used in the sequencing reactions to read both DNA strands. Sequences were edited and assembled using MEGA4 (Tamura and others 2007) and then subjected to GenBank BLASTN to identify them by sequence homology with described yeast taxa.

The sequences of the D1/D2 26S rRNA gene of isolates identified as *Clavispora lusitaniae* and closely related taxa retrieved from BLASTN search were included in multiple alignments generated using MEGA4 (Tamura and others 2007). The Kimura 2-parameter model was used for distance correction, and the Neighbor-Joining method (Saitou and Nei 1987) was used for phylogenetic inference. Support for tree branches was evaluated by bootstrap analysis from 1000 heuristic searches. Sequences AJ539366 and U44818, representing the D1/D2 26D rRNA of *Candida fructus* CECT 11884T, and *Clavispora opuntiae* NRRL Y-11820, respectively, were used as tree outgroups.

Determination of the moisture and salt contents and pH of dairy products

The moisture content of examined dairy samples was analyzed using a vacuum oven as described by Bradley and Vanderwarn (2001). Salt content was measured using a Corning Chloride Analyzer 926 (Ciba Corning Diagnostics, Medfield, Mass., U.S.A.), based on the Volhard test (Marshall 1992). pH was measured using Corning 240 pH meter (Corning, Suffolk, U.K.).

Results and Discussion

Diversity and total counts of yeasts in dairy products

Forty yeast cultures were isolated from traditional Egyptian dairy products including fresh Domiati cheese, stored Domiati cheese, Kariesh cheese, and Matared cream. The yeast isolates were identified by RFLPs of the ITS-5.8S rDNA region (Esteve-Zarzoso and others 1999) and sequencing of the domains D1 and D2 of the 26S rRNA gene (Kurtzman and Robnett 1998). Table 1 shows the sizes of undigested PCR products and digested fragments of the 5.8S-ITS rDNA region as well as the sequence similarity (%) by BLASTN in GenBank of the D1/D2 26S rRNA gene sequences. The bands generated by RFLPs of the ITS-5,8S region were identical for the yeast isolates pertaining to the same species. The restriction patterns were matched to different yeast species using the www.yeast-id.com database.

Identification results showed that the isolated yeasts belonged to the species *Issatchenkia orientalis* (13 isolates), *Candida albicans* (4 isolates), *Clavispora lusitaniae* (*Candida lusitaniae*) (9 isolates), *Kodamaea ohmeri* (*Pichia ohmeri*) (1 isolate), *Kluyveromyces marxianus* (6 isolates), and *Candida catenulata* (7 isolates) (Table 1). These identified yeasts were recovered from samples of all examined products (Table 1). However, different degrees of yeast diversity were observed in these products. While fresh Domiati cheese

and Matared cream contained a single but different yeast species, stored Domiati cheese and Kariesh cheese contained diverse yeast floras consisting of 3 and 4 species, respectively. There were yeasts that commonly existed in different products such as *I. orientalis*, *K. marxianus*, and *Cl. lusitaniae* (*Candida lusitaniae*) (Table 1). However, *C. albicans* and *K. ohmeri* (*Pichia ohmeri*) were only detected in stored Domiati cheese and *Candida catenulate* was only detected in Kariesh cheese. Total yeast counts in examined dairy products were generally higher than 10^3 CFU/g, but lower than 10^5 CFU/g (Table 2). Kariesh cheese showed the highest total count of yeasts (>10^4 CFU/g) with the rest of examined products containing from 10^3 to 10^4 CFU/g.

Kariesh cheese is prepared by natural fermentation of milk (Abou Donia 1991). Raw milk is left without disturbance for 1 to 3 d at room temperature in pre-washed, dried earthenware pots called "Matared." This allows natural fermentation of milk, whereas fat globules rise to the surface forming a cream layer. This cream, called "Matared" cream, is removed and domestically consumed, marketed or manufactured into butter (Kurmann and others 1992). The fermented milk, called "Laban Rayeb," could be also domestically consumed or used for the preparation of Kariesh cheese by placing it in a rural carpet with the addition of slight salt. Carpet is then closed and hanged for 1 to 2 d for whey drainage. The fact that raw milk could be used for the preparation of both "Matared" cream and Kariesh cheese using interrelated preparatory processes could explain the co-existence of *K. marxianus* in both products as shown in this study. *K. marxianus* is among the most predominant and important yeast species in raw milk (Fleet 1990). The ability of *K. marxianus* to ferment lactose and hydrolyze milk fat and proteins enable this yeast to grow in milk and dairy products (Roostita and Fleet 1996; Gadaga and others 2000). However, the yeast species *Candida catenulata*, *I. orientalis,* and *Cl. lusitaniae* (*Candida*

Table 2—Total yeast counts and chemical characteristics of examined traditional dairy products.[a]

Product	Total yeast count (CFU/g)	Moisture (%)	Salt (%)	pH
Fresh Domiati cheese	2350 ± 360	62.71 ± 2.6	4.65 ± 0.25	6.45 ± 0.10
Stored Domiati cheese	4000 ± 540	55.65 ± 1.2	8.12 ± 0.76	5.64 ± 0.10
Kariesh cheese	45000 ± 1050	72.50 ± 3.4	0.80 ± 0.05	4.21 ± 0.20
Matared cream	2600 ± 420	81.23 ± 2.2	0.11 ± 0.04	5.70 ± 0.25

[a]Data are presented as means of 3 replicates ± SD.

Table 1—Molecular characteristics and identification results of yeasts isolated from traditional dairy products.

Origin	Isolate	AP	ITS-5.8S RFLP (base pairs) CfoI	HaeIII	HinfI	GenBank acc. number	Species
Fresh Domiati cheese	FSMP Y1	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627964	*Issatchenkia orientalis*
	FSMP Y2	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627965	*I. orientalis*
	FSMP Y3	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627966	*I. orientalis*
Stored Domiati cheese	FSMP Y4	550	290 + 260	450 + 90	270 + 250	FJ627953	*Candida albicans*
	FSMP Y5	550	290 + 260	450 + 90	270 + 250	FJ627954	*C. albicans*
	FSMP Y6	550	290 + 260	450 + 90	270 + 250	FJ627955	*C. albicans*
	FSMP Y7	550	290 + 260	450 + 90	270 + 250	FJ627956	*C. albicans*
	FSMP Y8	400	200 + 90 + 90	400	200 + 190	FJ627984	*Clavispora lusitaniae*
	FSMP Y9	400	200 + 90 + 90	400	200 + 190	FJ627985	*Cl. lusitaniae*
	FSMP Y10	420	420	420	210 + 175	FJ627957	*Kodamaea ohmeri*
Kariesh cheese	FSMP Y11	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627967	*Issatchenkia orientalis*
	FSMP Y12	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627968	*I. orientalis*
	FSMP Y13	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627969	*I. orientalis*
	FSMP Y14	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627970	*I. orientalis*
	FSMP Y15	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627971	*I. orientalis*
	FSMP Y16	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627972	*I. orientalis*
	FSMP Y17	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627973	*I. orientalis*
	FSMP Y18	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627974	*I. orientalis*
	FSMP Y19	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627975	*I. orientalis*
	FSMP Y20	520	200 + 170 + 70	380 + 90	220 + 150 + 140	FJ627976	*I. orientalis*
	FSMP Y21	750	290 + 180 + 140 + 90	650 + 80	250 + 190 + 120 + 80	FJ627958	*Kluyveromyces marxianus*
	FSMP Y22	750	290 + 180 + 140 + 90	650 + 80	250 + 190 + 120 + 80	FJ627959	*K. marxianus*
	FSMP Y23	750	290 + 180 + 140 + 90	650 + 80	250 + 190 + 120 + 80	FJ627960	*K. marxianus*
	FSMP Y24	750	290 + 180 + 140 + 90	650 + 80	250 + 190 + 120 + 80	FJ627961	*K. marxianus*
	FSMP Y25	400	210 + 190	400	200 + 190	FJ627977	*Candida catenulata*
	FSMP Y26	400	210 + 190	400	200 + 190	FJ627978	*C. catenulata*
	FSMP Y27	400	210 + 190	400	200 + 190	FJ627979	*C. catenulata*
	FSMP Y28	400	210 + 190	400	200 + 190	FJ627980	*C. catenulata*
	FSMP Y29	400	210 + 190	400	200 + 190	FJ627981	*C. catenulata*
	FSMP Y30	400	210 + 190	400	200 + 190	FJ627982	*C. catenulata*
	FSMP Y36	400	210 + 190	400	200 + 190	FJ627983	*C. catenulata*
	FSMP Y31	400	200 + 90 + 90	400	200 + 190	FJ627986	*Clavispora lusitaniae*
	FSMP Y32	400	200 + 90 + 90	400	200 + 190	FJ627987	*Cl. lusitaniae*
	FSMP Y33	400	200 + 90 + 90	400	200 + 190	FJ627988	*Cl. lusitaniae*
	FSMP Y34	400	200 + 90 + 90	400	200 + 190	FJ627989	*Cl. lusitaniae*
	FSMP Y35	400	200 + 90 + 90	400	200 + 190	FJ627990	*Cl. lusitaniae*
	FSMP Y37	400	200 + 90 + 90	400	200 + 190	FJ627991	*Cl. lusitaniae*
	FSMP Y38	400	200 + 90 + 90	400	200 + 190	FJ627992	*Cl. lusitaniae*
Matard cream	FSMP Y39	750	290 + 180 + 140 + 90	650 + 80	250 + 190 + 120 + 80	FJ627962	*Kluyveromyces marxianus*
	FSMP Y40	750	290 + 180 + 140 + 90	650 + 80	250 + 190 + 120 + 80	FJ627963	*K. marxianus*

AP = size in base pairs of the ITS-5.8S rDNA PCR product.

lusitaniae) could be isolated from Kariesh cheese but not from "Matared" cream. This could be attributed to the further processing of "Laban Rayeb" used for the making of Kariesh cheese, which involves few days of hanging in rural carpets for whey drainage. Such conditions may allow additional yeasts to gain access to Kariesh cheese. Whereas, "Matared" cream is directly consumed or marketed following its separation from "Laban Rayeb." Interestingly, yeasts isolated from Kariesh cheese have been also recovered from different cheese types in Austria, Denmark, France, Germany, and Italy (Prillinger and others 1999) and from Gubben (a smear-red cheese) (Rea and others 2007). This suggests that these yeasts form a common flora in cheese.

Stored Domiati cheese came 2nd in yeast diversity after Kariesh cheese, containing a more diverse yeast flora than fresh Domiati cheese. Stored and fresh Domiati cheeses are prepared using the same procedures, but the major differences between them are the higher salt content of the stored Domiati cheese and the pickling of this cheese in brine solution or salted whey for a few months. This may explain the presence of more yeast species in stored Domiati cheese than in fresh Domiati cheese, given that brines are considered as a major source of yeasts in cheese (Seiler and Busse 1990; Kaminarides and Laskos 1992; Viljoen and Greyling 1995).

Among the yeast species found in stored Domiati cheese, it is important to highlight the presence of *K. ohmeri* (*Pichia ohmeri*) and *C. albicans*. Strains of the species *K. ohmeri* have only been previously found in Brazilian artisanal cheese (Borrelli and others 2006). However, this yeast species has been frequently isolated from other sources including the surface of cucumber brines (Deak and Beuchat 1996) and recently from marine fish (Li and others 2008). The presence of the pathogenic yeast *C. albicans* in stored Domiati cheese was also interesting. This pathogenic yeast has not been reported in other analyses of yeast populations in cheeses; however, another opportunistic pathogenic yeast, *C. tropicalis*, has been reported in Zimbabwean dairy products (Gadaga and others 2000), feta cheese (Tzanetakis and others 1998), and yogurt (Rohm and others 1992). The source of these pathogenic yeasts in dairy products is not well known, although their presence in cheese brines has been reported (Seiler and Busse 1990). This may explain the presence of *C. albicans* in samples of stored Domiati cheese examined in the present study. However, it may have been also transmitted from infected persons handling cheese during the preparation or marketing. Food products have generally been considered as insignificant transmission vehicles of pathogenic yeasts (Fleet 1990, 2007). However, Todd (1983) reported cases where yeasts were suspected of causing food poisoning and Taylor (1980) also described the possibility of yeasts being connected with allergic reactions of consumers to foods. The present study provides new evidence that stored Domiati cheese could act as a transmission agent of the pathogenic yeast *C. albicans*.

Yeasts have been reported to contribute to cheese ripening due to their lipolytic and proteolytic activities (Frölich-Wyder 2003). For instance, *C. catenulata* has been characterized by its strong extracellular proteolytic and lipolytic enzymes contributing to flavor development in cheese (Roostita and Fleet 1996). The ability of some yeasts to metabolize lactic acid and thus increase pH was also shown to aid the growth of certain acid-sensitive microorganisms involved in cheese ripening (Frölich-Wyder 2003). These yeasts involve *Cl. lusitaniae* and *C. catenulata* that were shown to lower the acidity of cheese curd slurries by the assimilation of lactic acid (Wyder and Puhan 1999). Moreover, the lactose fermenting species *K. marxianus* was reported to contribute to cheese flavor by its metabolic reactions leading to the production of ethanol and acetaldehyde (Devoyod 1990). Within this context, a role of yeasts isolated in this study in the formation of characteristic flavors of the examined Egyptian cheeses could be expected. This could be more relevant to stored Domiati cheese that is preserved in brine solution for months for the development of its typical flavor. However, further studies will be needed to experimentally address this aspect.

Chemical characteristics of dairy products

To examine whether there was a relationship between yeast counts and diversity, and certain chemical characteristics of the examined products, samples of dairy products were analyzed for their content of moisture and salt and pH values. Table 2 shows that Matared cream and Kariesh cheese had generally higher moisture contents than fresh and stored Domiati cheese. Stored Domiati cheese showed the highest salt content, followed by fresh Domiati cheese, but Kariesh cheese and Matared cream contained relatively low salt contents. pH values were generally acidic and ranged from 4.21 to 6.45 in examined samples. Kariesh cheese was the most acidic followed by stored Domiati cheese, with fresh Domiati cheese having slightly acidic pH.

The chemical characteristics of these products could generally aid the growth and survival of yeasts. In particular, low pH in fermented milks provides a selective environment for yeast growth, but is unfavorable for most bacteria (Fleet 1990; Rohm and others 1992; Deak and Beuchat 1996). That Kariesh cheese had the highest moisture content, low salt content, and the most acidic pH, this could explain its containing the most diverse yeast floras and highest yeast counts among other products.

Molecular characterization of *Cl. lusitaniae* isolated from dairy products

The D1/D2 26S rRNA gene sequences were 100% identical for the yeast isolates pertaining to the same species except for the isolates of the species *Cl. lusitaniae*. These isolates were recovered from stored Domiati cheese (2 isolates) and kariesh cheese (7 isolates) (Table 1). Phylogenetic reconstruction based on the D1/D2 26S rRNA gene sequences of these 9 isolates grouped them in 3 clusters (Figure 1). Cluster 1 involved 2 isolates from stored Domiati cheese (FSMP Y8 and FSMP Y9) and 3 isolates from kariesh cheese (FSMP Y34, FSMP Y32, and FSMP Y35). Whereas, cluster 2 was represented by strain CBS 4413 and 3 isolates from kariesh cheese (FSMP Y37, FSMP Y38, and FSMP Y31), and cluster 3 included strain CBS 6936^T and 1 isolate from kariesh cheese (FSMP Y33). The D1/D2 26S rRNA gene sequences of strains grouped in clusters 1 and 2 differed in 2 nucleotide substitutions in positions 457 (T to C) and 492 (T to C). The D1/D2 26S rRNA gene sequence of strains in clusters 1 and 2 differed from the CBS 2866 sequence by 2 nucleotide substitutions in positions 177 (A to C) and 178 (T to C). The D1/D2 26S rRNA gene sequence shared by the type strain CBS 6936^T and isolate FSMP Y-33 (cluster 3) differed by more than 30 base substitutions from the sequence type found in the above *Cl. lusitaniae* cheese strains. These sequence substitutions were located in a hypervariable region of 90 nucleotides approximately between positions 406 and 496 of the D1/D2 26S rRNA gene sequence.

The heterogeneity in the D1/D2 26S rRNA gene of *Cl. lusitaniae* isolates as shown in this study was not unexpected. This phenomenon was previously reported by Lachance and others (2003) who found 10 different versions of the D1/D2 26S rDNA sequence within 38 strains of this yeast isolated from different sources. The variations in the D1/D2 26S rRNA gene sequence displayed by the 9 cheese isolates of *Cl. lusitaniae* in the present study were similar to those described by Lachance and others (2003). Cluster 3 corresponded to Lachance's type H1, whereas cluster 2 corresponded to Lachance's type I1. However, there were new strains in cluster 1

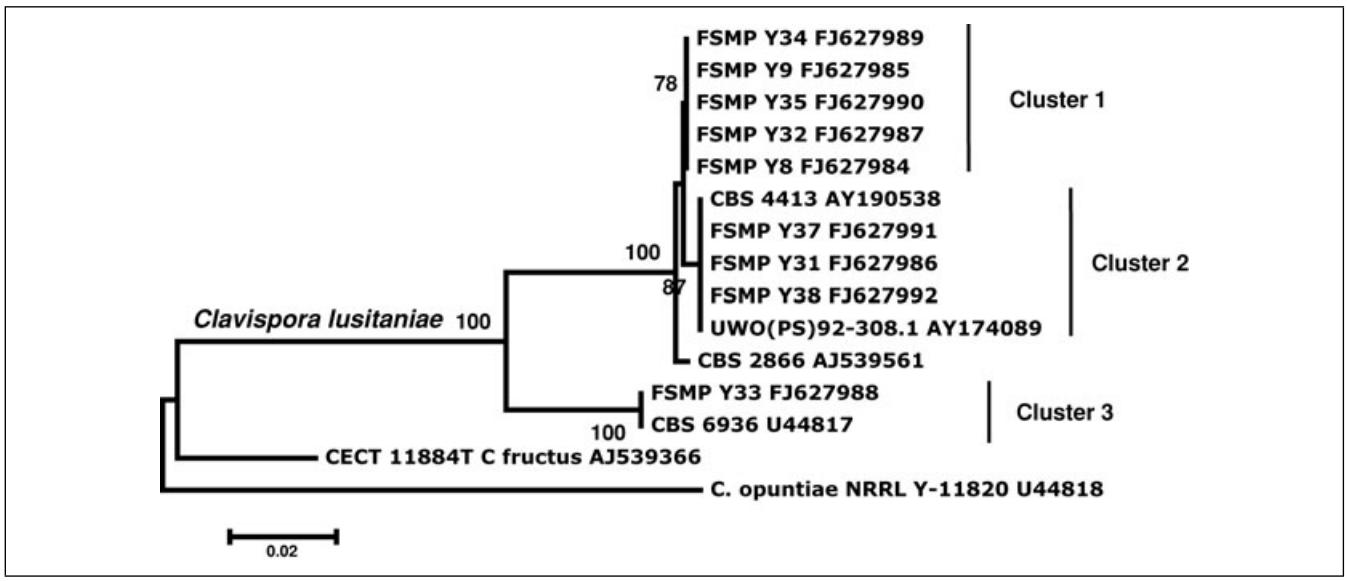

Figure 1 — Neighbor-Joining phylogenetic tree of the polymorphic sequences of the D1/D2 domains of the 26S rDNA of *Clavispora lusitaniae*.

displaying a new sequence type between Lachance's types I1 and I3. On the other hand, previous studies on the heterogeneity of the 26S rDNA sequence in other yeast species, such as *C. albicans*, *C. dublinensis,* and *P. guilliermondii* revealed very few nucleotide substitutions among strains pertaining to the same species (Kurtzman and Robnett 1997). This is consistent with the present study, where the D1/D2 26S rRNA gene sequences were 100% identical for all isolates pertaining to the same species, but those of *Cl. lusitaniae*.

Conclusions

The present study shows that diverse yeast species existed in the examined traditional Egyptian dairy products with Kariesh cheese containing the most diverse yeast flora. The level of yeast diversity was found to correlate with the chemical characteristics of the examined products in terms of moisture content, salt content, and pH. While most of the isolated yeasts had been also isolated in other food products in other world areas, this study reports the 1st incidence of the pathogenic yeast *C. albicans* from dairy products. Another interesting finding was the identification of diverse *Cl. lusitaniae* isolates associated with stored Domiati cheese and kariesh cheese. This provided another line of evidence on the diversity of strains within this yeast species.

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