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Studies on Beneficial Yeasts Isolated from Some Egyptian Dairy Products

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Abstract

Yeasts constitute large groups of microorganisms that are recently attracting attention of industry and scientists. Different biological activities make them favorable candidates for numerous applications not limited to the food sector. The aim of this study was to examine beneficial yeasts associated with some traditional Egyptian dairy products and to evaluate their microbiological and technological properties. One hundred samples of locally produced yoghurt, Kariesh, Domiatti and Mish cheeses were collected from different localities at Sharkia Governorate, Egypt. Seventy-seven (77%) of the examined samples were positive for the presence of yeasts. Kariesh cheese had the highest total yeast count compared with other examined dairy products. From these positive samples, a total of 154 yeast isolates were screened for their antibacterial activities against the common foodborne pathogens (*Staphylococcus aureus* and *Escherichia coli*). Yeast isolates showing highest antibacterial activity were identified and then investigated for some technological properties (growth ability at different temperature, low pH and different NaCl conditions). Only 30 strains corresponding to *Saccharomyces cerevisiae*, *Debaryomyces hansenii* and *Candida zeylanoides* were found to be effective against *S. aureus* and/ or *E. coli* strains. These strains have also technological properties that allow them attractive for use in food industry and for further evaluation.

Keywords: Yeasts, Antimicrobial activity, Probiotic, Technological Properties.

Introduction

Yeasts are unicellular eukaryotic fungi that are widely present in natural environments such as soil, plants, water, fruit juice, leaf surface, milk and intestinal tract of warm blooded animals [1]**.** Yeasts can contaminate food during food processing from various sources such as the floor, air, wall, hands, equipment and apron [2]. According to the ability of yeasts to tolerate the low pH, high osmotic condition and low refrigeration temperatures, they can cause spoilage and changes in organoleptic, physical and chemical properties of foods (such as: off-flavor formation, gas production and discoloration) [3]. In contrast, yeasts have the ability to play very essential roles in the production of some fermented foods and contribute to their aroma and flavor [4]. Also, they constitute an essential part of the micro-flora of many types of cheese because of the high salt concentration, low pH and low moisture content of these products [5]. Similarly, yeasts can eliminate or inhibit undesirable microorganisms during fermentation because of their antifungal and antibacterial activities [6].

The inhibitory activity of yeasts associated with fermented foods is mainly due to competition for nutrients, changes in pH of the medium due to production of organic and volatile acid, high concentrations of ethanol, hydrogen peroxide and antimicrobial compounds such as killer toxins or "mycocins" [7,8]. Because of their antagonistic action against pathogenic microorganisms, they had been used as biocontrol agents and some yeasts were considered as probiotic organisms [9]**.**

Probiotics were defined according to FAO/WHO as live microorganisms which, when administered in certain amounts, confer health benefits to the host [10]**.** Yeast strains' characters including antagonistic action against foodborne pathogens and the ability to

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resist acid and bile conditions may contribute to the production of fermented products with potential probiotic properties [11]. Among the yeast species in dairy products, *Saccharomyces cerevisiae* (*S. cerevisiae*) was found to be the predominant and the most significant species [12,13].

Many scientists have focused mainly on *Lactobacillus* and *Bifidobacterium* for food containing probiotics. Whereas, the yeast *S. cerevisiae* has been known to be effective for treatment of gastroenteritis and has the potential to be used as probiotics [14,15]. Using probiotic *Saccharomyces* species alone or in combination with lactic acid bacteria can enhance the nutritive value of fermented dairy products [16]. The potential probiotic effect of *S. cerevisiae* has been demonstrated due to its ability to tolerate low pH and bile resulting in protection against bacterial infection [17].

A general protocol proposed by Pulvirenti *et al.* [18] could be used for the selection of functional yeasts (probiotic or starter). One of the most important characteristics of this kind of yeasts should be technological feasibility including; growth under various conditions of temperature, pH and salt [19].

The objectives of this study were to isolate and identify yeasts of high antibacterial activity, and to characterize some technological properties (pH, NaCl and temperature resistance) of yeasts isolated from different traditional Egyptian dairy products.

Material and Methods

Collection and preparation of the samples

A total of 100 samples of traditional and locally produced Egyptian dairy products (yoghurt, Kariesh, Domiatti and Mish cheese, 25 each) were collected from different localities at Sharkia Governorate during January-June, 2015, and sent immediately to the laboratory for examination. For yoghurt, 1 g of each mixed sample was added to 9 mL of sterile 0.1% peptone water while for Kariesh, Domiatti and Mish cheese, 11 g of each sample were mixed thoroughly with 99 mL of sterile 2% sodium citrate solution in a pre-warmed sterile mortar (40°C) till complete emulsification, followed by 1:10 serial dilution [20].

Enumeration and isolation of yeasts

From each dilution, 0.1 mL of the suspension was transferred to Yeast Peptone Dextrose (YPD) agar plates (HiMedia) containing 10 mg/mL of ampicillin. The plates were incubated at 25°C for 3-5 days. From each countable plate, representative colonies were picked up and streaked to obtain a pure culture for further examination. Isolates were maintained at -20°C in YPD broth (HiMedia) with 20% (v/v) glycerol [21].

Antibacterial activity

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* serotypes (*E. coli*) were used as indicator bacteria. Pure cultures of both pathogens (kindly obtained from the Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University) were inoculated into nutrient broth and incubated at 37^{o}C for 24 h [22]. Antibacterial activities of yeast isolates were determined by Agar Well Diffusion method by preparation of Muller-Hinton agar (Himedia) plates. The wells were made and filled with 100 µL of yeast culture supernatant which was made by inoculating isolated yeast strains in YPD broth and incubated at 25^{o}C for 3 days followed by filtration. The inoculated plates were incubated at 37^{o}C for 24 h [23].

Identification of yeast isolates with antibacterial effect

Identification of each yeast isolate that had antibacterial activity into genera and species level was carried on the basis of standard morphological, physiological and biochemical tests [24,25].

Characterization of some technological properties

The growth ability of yeasts at different temperatures, different NaCl concentrations and at low pH was determined. Tubes containing YPD broth were inoculated with 1% activated cultures of yeast isolates and incubated at 10°C and 37°C for 3-5 days. Growth of yeasts was determined by visual observation. While in order to detect yeasts tolerance to NaCl, activated cultures were inoculated in YPD tubes adjusted to 10 and 20% NaCl. The tubes were incubated at 26°C for 3-5 days and the growth was determined visually. To estimate growth abilities of yeasts at low pH, activated cultures (1%) were inoculated into YPD tubes with pH adjusted to 2.5 and 3.5 (with 3N HCl). The tubes were then incubated at 26°C for 3-5 days and the results were determined visually. The experiments were conducted in duplicate with two independent occasions [26].

Results and Discussion

Data presented in Table (1) revealed that yeasts were detected in 100, 92, 76 and 40% out of the examined Kariesh cheese, yoghurt, Domiatti and Mish cheeses samples, respectively. Results recorded previously revealed that all the examined Kariesh cheese, Domiatti cheese and yoghurt samples were contaminated by yeasts with the percentages of 100, 100, 50%, respectively [27]. Moreover, several reports have also detected yeasts at varying percentages in different traditional dairy products [28-30].

There was variation in the count of yeasts between different dairy products under study. In general, yeasts were present in all Kariesh cheese samples with the highest count among the examined samples (a minimum of 5.9×10^4 and a maximum of 1.3×10^7 CFU/g with a mean of 3.5×10^6 CFU/g) (Table 1).

Table 1: Yeast counts/g in the examined traditional Egyptian dairy products collected from different localities at Sharkia governorate during January-June, 2015

Type of product	No. of samples	Positive Samples No.	Positive Samples %	No. of yeast isolates	Counts/ g Min.	Counts/ g Max.	Counts/ g Mean ±S.E.M.
Locally produced yoghurt	25	23	92.0	46	2.0×10^3	1.5×10^5	$4.2\times10^4\pm0.8\times10^4$
Kariesh cheese	25	25	100.0	50	5.9×10^4	1.3×10^7	$3.5\times10^6\pm0.7\times10^6$
Domiatti cheese	25	19	76.0	38	1.4×10^4	5.8×10^6	$8.0\times10^5\pm3.4\times10^5$
Mish cheese	25	10	40.0	20	3.0×10^3	1.3×10^5	$3.1\times10^4\pm1.2\times10^4$

Production of Kariesh cheese depends on natural fermentation of raw milk [31]. The possible explanation of the higher isolation rate and count of yeasts in Kariesh cheese compared to the other cheese types could be that Kariesh cheese is made without heat treatment and thus giving opportunity to yeasts to grow and ferment lactose and hydrolyze milk fat and proteins enabling them to grow in milk and dairy products [32,33].

The average counts of yeasts in locally produced yoghurt samples was $4.2\times10^4\pm0.8\times10^4$ CFU/g with a minimum of 2.0×10^3 and a maximum of 1.5×10^5 CFU/g. While in Domiatti cheese samples, the mean count was $8.0\times10^5\pm3.4\times10^5$ with a minimum of 1.4×10^4 and a maximum of 5.8×10^6 CFU/g. Other reports revealed that Kariesh and Domiatti cheese samples were contaminated by yeasts with lower values with log mean values of 4.5±0.4 and 4.3±0.2 CFU/g [27]. In contrary, higher findings were reported in yoghurt samples by Abu-Elreesh [34]. According to the Egyptian Organization for Standardization and Quality Control (EOS) [35], Kariesh cheese and Domiatti cheese should not contain more than 400 CFU/g of total yeasts, while yoghurt should be free from yeasts. The obtained results of the current study were higher than the permissible values recommended by EOS (Table1).

The explanation of locally produced yogurt contamination could be the manufacture of yogurt at home using starter culture from previous batches of yogurt which might be contaminated over time. In addition, yoghurt is containing sugar and moisture which are essential ingredients for yeast development.

The lowest yeast count was in Mish cheese samples with a minimum of 3.0×10^3 and a maximum of 1.3×10^5 CFU/g. This low count could be due to the high salt concentration in Mish which was not suitable for their growth [36]**.** Lower results of yeast count in Mish cheese were reported by Sulieman [36], while, Osman [37] could not detect yeast cells in Mish cheese samples.

Table 2: Frequency distribution of the examined traditional Egyptian dairy products based on their yeast counts /g

Intervals CFU/g	Yoghurt		Kariesh cheese		Domiatti cheese		Mish cheese	
	No.	%	No.	%	No.	%	No.	%
10^3-10^4	5	21.7	0	0	0	0	4	40
10^4-10^5	15	65.2	1	4	5	26.3	5	50
10^5-10^6	3	13.1	4	16	12	63.2	1	10
10^6-10^7	0	0	19	76	2	10.5	0	0
10^7-10^8	0	0	1	4	0	0	0	0
Total	23	100	25	100	19	100	10	100

The differences in microbial counts between the present study and other studies could be attributed to the origin of the samples or time of samples collection [36].

The highest frequency distribution of the examined yoghurt samples for the yeast count lies within the range 10^3 - $<10^5$ CFU/g (86.9%) (Table 2). While the highest frequency distribution of examined Kariesh, Domiatti and Mish cheese samples for the yeast count lies within the range 10^5- $< 10^7$ (92 %), 10^4 - $<10^6$ (89.5 %) and 10^3- $<10^5$ (90%), respectively.

The results of yoghurt are nearly similar to the results recorded by Abdel-Fatah [38] who showed that 77.8% of the examined yoghurt samples had yeast counts within the range of 10^3-10^5 CFU/g. While the results of Kariesh cheese were in contrast with those obtained by El-Diasty and Salem [39] who reported that the highest frequency distribution of the examined cheese samples lies within the range 10 – 100 CFU/g.

Table 3: Proportion of yeast isolates showing antibacterial activity in the examined samples

Type of product	No. of samples	No. of Yeast isolates	Overall yeast isolate with antibacterial activity	Yeast isolates having antibacterial effect against *S. aureus* and/or *E. coli*			
				S. aureus		*E. coli*	
				No.	%	No.	%
Yoghurt	25	46	2	0	0	2	4.3
Kariesh cheese	25	50	13	4	8	12	24
Domiatti cheese	25	38	6	6	15.8	0	0
Mish cheese	25	20	9	4	20	5	25

Table (3) shows the proportion of yeast isolates recovered from the examined products. A total of 154 yeast isolates (46, 50, 38 and 20 colonies from yoghurt, Kariesh, Domiatti and Mish cheeses samples, respectively) were isolated and screened for their antibacterial activities.

Table 4: Antibacterial effect of yeast isolates recovered from Traditional Dairy Products having antibacterial activity using *S. aureus* and *E. coli* as indicator bacteria

Types of product	Isolate code	Presence or absence of inhibition zones		Isolate code	Presence or absence of inhibition zones		Isolate code	Presence or absence of inhibition zones	
		S. aureus	*E. coli*		*S. aureus*	*E. coli*		*S. aureus*	*E. coli*
Yoghurt	**6**	-	+	**8**	-	+			
	5	-	+	**15**	-	+	**22**	-	+
	6	+	-	**16**	+	+	**23**	-	+
Kariesh cheese	**8**	-	+	**18**	-	+	**25**	-	+
	9	+	+	**19**	-	+			
	9	-	+	**20**	+	+			
Domiatti cheese	**3**	+	-	**13**	+	-	**16**	+	-
	6	+	-	**14**	+	-	**24**	+	-
	1	-	+	**13**	-	+	**21**	+	-
Mish cheese	**2**	+	-	**15**	-	+	**22**	-	+
	3	+	-	**16**	+	-	**23**	-	+

-: no inhibition +: The inhibition zone > 5 mm is considered positive

Thirty isolates were able to inhibit the growth of *S. aureus* and/or *E. coli* (Table 4). Only three yeast strains inhibited the growth of both *S. aureus* and *E. coli* while the inhibitory effect of other yeast strains varied against pathogenic strains, and appeared to be a strain-dependent. Yeasts are useful for biocontrol purposes against foodborne pathogens because they are mostly non-pathogenic and do not produce mycotoxins or allergenic spores [40]. Some yeasts are desirable as starter adjuncts in order to inhibit wild strains that cause problems in the food industry. The possible reasons for these activities are competition for nutrients, secretion of cell wall degrading enzymes and the production of killer toxins [41].

Historically, the first positive antagonistic activity of yeasts was published early in the twentieth century by Hayduck [42] who reported that yeasts could inhibit the growth of *E. coli* and staphylococci [43]. Rajkowska *et al.* [10] reported a reduction in the number of *S. aureus* cells after co-incubation with probiotic yeasts. Also, Bornet and Bergogne-Berezin [44] observed a decrease in bacterial counts of *S. aureus* in the presence of *S. cerevisiae.* Some strains of candida species showed various antibacterial activities towards pathogenic bacteria such as *E. coli* and *S. aureus* [6]. *Debaryomyces hansenii* was the most common yeast species found in cheese and could produce killer toxins inhibiting food-borne pathogens [45].

A total of 30 yeast isolates that showed antibacterial activity were identified in this study on the basis of morphology, physiological and biochemical tests. The results showed that yeast isolates belonged to *S. cerevisiae* (3 isolates), *D. hansenii* (16 isolates) and *C. zeylanoides* (11 isolates).

Table 5: The technological properties of identified yeast strains

Yeast strains	**Growth at**					
	Temperature		**NaCl (%)**		**pH**	
	10°C	37°C	10%	20%	pH 2.5	PH 3.5
***S. cerevisiae* (n=3)**	+	-	+	-	-	+
***D. hansenii* (n=16)**	+	-	+	+	-	+
***C. zeylanoides* (n=11)**	+	+	+	-	+	+

- (No activity) + (activity)

To determine the technological properties of these identified yeasts, their growth under different conditions (temperature, NaCl and pH) was determined. All yeast strains could grow at 10°C but only *C. zeylanoides* could grow at 37°C (Table 5). These results are similar to those previously reported by Ozturk [46]. Another study reported by Ozturk [47] reported that four strains of *C. zeylanoides* could grow at 35°C. Some *C. zeylanoides* strains that could grow at 35°C might create a risk in products consumed without any heat treatment due to their opportunistic behaviors. *S. cerevisiae* could grow at a wide range of temperature [48]. The effect of temperature (10, 15 and 37°C) relied upon the isolate type as only some yeasts were able to grow at 10 or at 37°C or both of them.

All yeast species were resistant to 10% NaCl while no growth was observed at 20% NaCl concentration for all yeast isolates except for *D. hansenii* which could grow (Table 5). This was also found in other reports [46]**.**

D. hansenii is widely present in nature and common in cheeses and food products containing high salt concentrations [49-51]. It was the predominant in different types of cheese due to its ability to grow in the presence of high salt [52] and low pH [53]**.** It could grow at high osmotic concentrations up to 25% NaCl [54]**.** Effect of salt on yeast growth is strong and dose-dependent [48].

Some *S. cerevisiae* isolates were affected by NaCl 6%. Also, it was observed that *C. zeylanoides* and *S. cerevisiae* strains could grow at pH 2.5 and 3.5, but D. *hansenii* strains were capable of growing only at pH 3.5 (Table 5). Similar results were obtained by Ozturk [46] for C. *zeylanoides* and *D. hansenii.* Perricone *et al.* [48] recorded similar results for *S. cerevisiae* strains.

Conclusion

In conclusion, some yeast species can be used in processing due to their technological properties as growth at different temperature, NaCL concentration and low pH. In addition, some yeasts had antibacterial activity against food-borne pathogens.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي

دراسات عن الخمائر النافعة المعزولة من بعض منتجات الألبان المصرية

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تشكل الخمائر مجموعات كبيرة من الكائنات الحية الدقيقة التي جذبت انتباه صناعة الغذاء والعلماء حديثا . ولامتلاكها العديد من الأنشطة البيولوجية المختلفة أصبحت مناسبة للعديد من التطبيقات. لذا كان الهدف من هذه الدراسة فحص الخمائر المرتبطة ببعض منتجات الألبان المصرية المصنعة محليا وتقييم بعض الخصائص التكنولوجية للعزلات التي لها القدرة علي مقاومة البكتريا المسببة للامراض. ولقد أوضحت هذه الدراسة أن الخمائر متواجدة بنسبة ٧٧% من عينات الزبادي، الجبن القريش، الجبنة الدمياطي والمش المنتجة محليا في مناطق مختلفة بمحافظة الشرقية وكانت أعلى نسبه خمائر بعينات الجبن القريش مقارنة مع غيرها من منتجات الألبان. ولقد تم فحص ١٥٤ عزلة من الخمائرة لاختبار نشاطها ضد مسببات الأمراض التي من أهمها المكورات العنقودية الذهبية و الإيشريشيا القولونية. كما تم فحص بعض الخصائص التكنولوجية للعزلات التي كان لها أعلى نشاط مضاد للجراثيم مثل القدرة علي النمو في درجات حرارة مختلفة، وانخفاض درجة الحموضة و كلوريد الصوديوم بتركيزات مختلفة). ولقد اوضحت النتائج ان ٣٠ عزلة فقط قد أثبتت نشاط مقاومة مسببات الامراض. وتتمتع هذه العزلات بخصائص تكنولوجية تجعلها تجذب الانتباه لاستكمال دراستها واستخدامها في صناعة الغذاء.