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Characterisation of Yeasts Isolated from Artisanal Turkish Dairy Products

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Abstract: Economic and sensorial losses of dairy products due to spoilage by yeast have been increasing in Turkey because of poor hygienic conditions during processing and to shorten the anticipated shelf life of products and/also reduced use of preservatives that do not strictly control the growth of these organisms. This study reports the results of a survey of yeast species in samples of cow's, ewe's and goat's milk products collected in some regional bazaar of Izmir, Western Turkey. Yeasts were isolated from white pickled cheese, tulum cheese and kashkaval cheese, yoghurt and strained yoghurt respectively. The most frequently occurring yeast species on dairy products were *Candida* sp., *Saccharomyces* sp., *Kluyveromyces* sp. and *Trichosporon* sp. respectively. Even though yeasts are considered as environmental contaminants, the occurrence of some of them in dairy products at high levels could represent a potential risk for human health especially for immuno compromised patients.

Key words: Yeasts, identification spoilage, dairy products

Introduction

The main group of microorganisms generally associated with dairy products is covered by lactic acid bacteria although recently, it is well recognized that yeasts isolated from especially cheese and functional dairy products play a significant role in their ripening and developing some aroma components (Jacobsen and Narvhus, 1996; Pereira-Dias *et al*., 2000; Fadda *et al*., 2001; Minervini *et al*., 2001). Yeasts in dairy products may interact with other microorganism in three different ways:

- They may inhibit or eliminate microorganisms which are undesired because they cause quality defects or potentially pathogenic characters
- They may inhibit the starter culture activity or
- They may positively associate with the fermentation or maturation process by supporting the function of the starter culture (Jacobsen and Narvhus, 1996; Loureiro and Querol, 1999).

On the contrary, yeast can have a negative effect as spoilage organisms in fermented milk products and cheeses (Pereira-Dias *et al*., 2000; Minervini *et al*., 2001). The main defects caused by spoilage yeasts are fruity, bitter or yeasty off-flavours, gas production, discoloration changes and a softening of product texture (Corbo *et al*., 2001). Whereas, the use of yeasts in dairy industry could determine potential advantages covering production of flavour components and acceleration of ripening process of some cheese species by means of its lipolytic and proteolytic properties, fermentation ability of lactose, assimilation of lactate and

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positively interactions with the starter culture (Jacobsen and Narvhus, 1996; Fleet and Mian, 1987; Fleet, 1990; Roostita and Fleet, 1996).

In this study, we were undertaken to investigate the frequency of occurrence and types of yeasts associated with different animal origin dairy products located in some regions of Izmir.

Materials and Methods

The study was carried out on 106 samples of dairy products, representing manufactured from usually cow's milk and cow's, ewe's and goat's milk mixture. The samples were collected from grocery stores and bazaar in Izmir and Aydin two regions of western Turkey. Samples originated from different animal origin were distributed as follows according to type of dairy products:

- Forty nine different time ripened products; 15 white pickled cheese, 15 kashkaval cheese, 19 tulum cheese canned and strained
- Fifty seven unripened dairy products (no ripening required) 30 strained yoghurt, 27 set type yoghurt. Also quide by date coding we chose for analysis only yoghurt and stained yoghurt samples that had not been manufactured more than 5 days previously.

The samples were transported in an ice box to the laboratory where they were immediately analyzed (Suriyarachi and Fleet, 1981).

Identification of Yeasts

Yeasts isolation was performed according to ISO 8261. From each sample, 20 g of the different dairy products including yoghurt, strained yoghurt and cheese species were diluted in 180 mL peptone-water and homogenized in Stomacher Lab-Blender 400. Ten fold dilutions were prepared in quarter strength ringer's solution and suitable aliquots were plated on yeast glucose agar (Merck 0500 KgaA Darmsadt) with chloramphenicol (100 mg L^{-1}) added and plates were incubated at 25±1°C for 3-5 days. For the identification, at least three colonies of each different morphological type were selected from primary cultures. After purification on yeast glucose agar, the yeasts were grown on the same media and kept at 4°C until they were identified.

The colonie isolates were characterized to (Vander and Yarrow, 1984). Identification was carried out by comparing the test datas with (Lodder, 1970). Microscopic observations of cell morphology and the production of ascospores, arthrospores and true hyphae were used to supplement of biochemical tests (Deak, 1986; Deak and Beuchat, 1987).

Results and Discussion

Yeasts in fermented dairy products and especially cheese varieties are assumed as insignificant at the earlier stages of storage life, but are important component of the microflora of many, if not all, cheese varieties and some fermented products in later stages, being present as natural contaminants in the curd and the end product during maturation and storage (Corbo *et al*., 2001; Welthagen and Viljoen, 1998). The environment during ripening of the cheese and storage of fermented products consist of a low pH, moisture content, elevated salt concentration and low temperature which are often very favorable and selective for yeast growth (Vanden and Jakobsen, 1998; Viljoen and Greyling, 1995; Wojtatowicz *et al*., 2001). The increase in the number of yeasts corresponds with the deplation in lactose content and the simultaneous stabilization of lactic acid bacteria numbers used as a starter (Jacobsen and Narvhus, 1996; Fleet, 1990; Gadaga *et al*., 2000).

Table 1: Properties of yeast species which exhibited growth in cheese species

Species identified	No. of species	Fermentation Gl.	Ga.	L.	M	S.	Assimilation Gl.	Ga.	L.	M	S.	R.	Ra.	D.	N.	L.A.	KNO_3	J.	P.	A.	Morphologic characteristics .C.C.	Pi.	F.	T.	Se.	Gr. at 37°C
C. krusei	27	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	+	-	+	Clear-cream	-	-	V	+	+
C. kefir	22	+	+	V*	-	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+	Clear-cream	-	-	-	+	+
C. intermedia	14	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	Clear-cream	-	-	+	+	+
C. tropicalis	13	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	-	Clear-cream	-	+	+	+	+
C. fomata	11	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	Clear-cream	-	-	V	-	+
C. versatilis	9	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	-	+	Clear-cream	-	+	+	+	+
C. lipolytica	10	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+		+		-	-	+	V	+
T. cutaneum	17	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+	-	+		-	White-grey	-	-	+	V	+
T. eriense	7	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+		-	Yellow-grey	-	+	-	-	V
K. lactis	21	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+		+	White-grey	-	+	+	+	+
D. hanseni	25	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	Yellow-White	V	+	-	+	+
T. delbrueckii	6	V	+	+	+	V	V	+	+	V	+	+	V	+	+	V	-	+	-	+	White	-	V	-	-	-
S. roseus	2	+	V	+	V	+	+	V	+	+	+	V	-	-	+	+	V	+	+	-	Cream	-	V	+	-	V
S. cerevisiae	16	+	+	V	+	+	+	+	+	V	+	+	+	+	V	+	+	+	-	+	White	-	+	+	+	+
G. candidum	16	+	+	V	V	+	+	+	V	V	+	+	-	-	+	+	+	+	+	+	White	+	V	V	+	+
R. glutinis	7	+	+	+	V	+	+	V	+	+	V	+	+	-	+	-	+	+	-	-	Yellow-Orange	+	+	+	+	+
P. anemola	5	-	-	-	-	V	+	+	-	-	-	-	-	-	-	-	+	+	+	+	White-yellow	-	+	+	+	+

*V: Variability, Gl.: Glucose; Ga.: Galactose; L.: Lactose; M.: Maltose; S.: Saccharose; R.: Raffinose; Ra.: Ramnose; D.: Dextrane; N.: Nicine; L.A.: Lactic Acide; J.: Jelatine; P.: Pseudomicelyum; A.: Ascospore; C.C.: Colony Coleur; Pi.: Pigmentatiton; F.: Films/surface growth; T.: Turbidity; Se.: Sedimentation; Gr. at 37°C: Growth at 37°C

Cheese Species

As shown in Table 1, from different cheese varieties we isolated from a taxonomic point of view, 17 species belonging to 10 genera were identified.

Among to 16 species isolated from cheese varieties (white pickled, tulum, kashkaval) the most frequently developing yeasts belonged to the species *Candida* sp., *Debaryomyces* sp., *Kluyveromyces* sp., *Trichosporon* sp., *Saccharomyces* sp. and *Geotricum* sp. The frequencies of the other species in ranged between 3.1% and 0.88%. Results about the role of these species of yeasts in cheese varieties are confined and usually those reported adversely. It was reported that *Trichosporon* sp. caused formation of surface film on the cottage cheese (Brocklehurst and Lund, 1985) whereas *T. cutaneum* was also isolated from non spoiled Strancchini cheese, an Italian soft cheese (Corbo *et al*., 2001; Sarais *et al*., 1996). Also the source of isolation of *Trichosporon* sp. vary considerably, although many of them are of human and animal origin (Corbo *et al*., 2001). Occurrence of this species in high numbers could indicate undesirable hygiene and ineffective cleaning procedures (Corbo *et al*., 2001; Yamani and Abu-Jaber, 1994).

Candida sp. were also isolated especially from white pickled and tulum cheese but it was not possible to determine whether spoilage was connected with this species. *C. lipolytica* and *C. tropicalis* were also isolated in Kashkaval cheese and in strained tulum cheese Thus, the roles that yeasts play in ripening duration is not clear, but are probably associated with the capacity of lipolytic and proteolytic enzymes as well as ferment lactose, utilize lactic acid and grow at 4°C.

D. hansenii was the highly dominant species, being present in 25% of the samples and in very high numbers as well (10^5–10^9 cfu g^{-1}) other. Other frequently appearing species were *Kluyveromyces lactic*, *Saccharomyces cerevisiae* and *Pichia anemola*.

Especially *D. hansenii* and *C. catenulato* growth in white pickled cheese and tulum cheese are facilitated by its ability to assimilate. Lactose, lactic acid and citric acid to produce lipases and proteases and to resist to high salt concentrations and low pH values. On the basis of these characteristics it seems using a potential use of this yeasts species as a starter culture, as recommended by other scientists (Fadda *et al*., 2001; Welthagen and Viljoen, 1998; Vanden and Jakobsen, 1998; Fatichenti *et al*., 1983; Psomas *et al*., 2001; Fadda *et al*., 2004). The ability of *Kluyveromyces lactis* to assimilate and ferment lactose is assumed to be a key property contributing to its growth in cheese

Table 2: Properties of those yeast species that exhibited growth in yoghurt strained yoghurt samples

Species identified	No. of species	Fermentation				Assimilation											Morphologic characteristics			
		Gl.	Ga.	L.	M.	Ra.	Ga.	Tr.	In.	L.A.	Me.	S.	Ga.	L.A.	KNO_3	Ürea	P.	A.	G.in.B.	Gr. at 37°C
Saccharomyces cerevisiae																				
biovar I	17	+	+	V	+	+	+	V	-	-	V	+	+	+	-	-	-	-	Films+Turbidity	-
biovar II	5	+	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	V	Turbidity	-
biovar III	1	+	+	-	-	+	+	-	-	-	V	V	+	+	-	-	V	-	Films	+
P. farinosa	3	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+	-	Films+Turbidity	-
P. anemola	1	+	-	-	+	-	+	+	-	V	V	V	+	+	-	-	+	+	Films+Turbidity	+
C. blankii	1	-	-	-	+	+	+	-	V	-	-	+	+	+	-	-	+	-	Films+Slight turbidity	-
C. lipolytica	3	V	-	V	+	+	+	-	-	V	-	+	+	+	-	-	V	-	Films	-
C. tropicalis	2	+	V	V	+	V	V	V	-	V	-	+	V	+	-	+	+	V	Films	-
C. kefir	1	+	+	V	+	+	V	-	-	-	-	+	+	+	+	-	+	-	Films+Turbidity	-
K. marxianus var. lactis	5	+	+	+	-	+	+	-	-	+	-	+	+	+	-	-	+	-	Turbidity	+
K. marzianus var. marzianus	3	+	+	+	V	+	+	-	-	+	V	+	+	+	-	-	+	-	Films+Turbidity	V
G. candidum	7	+	-	-	-	-	+	V	-	-	-	+	+	+	-	-	V	+	Turbidity+White films	+
T. cutaneum	9	-	-	-	+	-	+	-	-	-	+	+	+	+	-	-	+	+	Turbidity+Sediment	-
T. brassicae	4	-	-	-	-	-	+	-	V	-	+	+	+	+	-	V	+	+	Turbidity+Sediment	-
D. hansenii	7	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	-	-	Films+Turbidity	+

*V: Variability, Gl.: Glucose; Ga.: Galactose; L.: Lactose; M.: Maltose; ; Ra.: Ramnose; Tr.: Trehalose; In.: Inocitol; ; L.A.: Lactic Acide; Me.: Mellibiose; S.: Saccharose; P.: Pseudomicelyum; A.: Ascospore; G.in.B.: Growing in Broth; Gr.at 37°C: Growth at 37°C

species, other fermented and unfermented dairy products (Fleet, 1990; Welthagen and Viljoen, 1998). In addition, the occupance of *Kluyveromyces lactis* during ripering is probably related to the ability of these species to metabolize milk protein and fat and in some cases lactic acid (Fleet and Mian, 1987).

The occupance of *Rhodotorula glutinis*, *Sporobolomyces roseus* in cheese has been reported previously (Fadda *et al*., 2001; Fleet and Mian, 1987; Vanden and Jakobsen, 1998; Viljoen and Greyling, 1995). These yeast strains are considered to be contributing as common air or natural contaminants in the cheese before they were stored, although *Rhodotorula* sp. are able to grow at sub-zero temperatures (Deak, 1986; Welthagen and Viljoen, 1998). Also these results are in agreement with previous reports (Minervini *et al*., 2001; Welthagen and Viljoen, 1998; Viljoen and Greyling, 1995). Moreover it can be said that, the presence some of large number of yeasts during the late stages of maturation suggests that some yeast species may play an important role in the ripening of cheese species especially tulum and Kashkaval cheese as observed in this study.

Yoghurt and Strained Yoghurt

Yeasts apparently are indigenous to yoghurt and strained yoghurt and their microbial flora form an interesting microbial ecosystem. The high acid content of yoghurts, which is enough to inhibite bacterial growth and the limited access of air to the yoghurt in the containers during cold storage make it a special microbial habitat that is most suitable for the growth of yeasts. Especially the high yeast counts in strained yoghurt produced by the traditional method directly after packaging suggest a high degree of contamination and mishandling of the product, as previously mentioned by Yamani and Abu-Jaber (1994). This problem occurred in yoghurt and skinned yoghurt samples could be associated to many factor such as the use of yoghurt starter cultures, yoghurt or both that were contaminated by yeasts or to the undesirable hygienic conditions prevailing during straining of the whey, especially resourced by the cleanliness of the bags and apparatus used. Lack of desirable hygienic practices during producing of fermented dairy products may also contribute to the contamination risk by yeasts.

Table 2 shows the yeasts found in yoghurt and strained yoghurt samples, the seven most commonly isolated species and the counts at which these species occurred. The isolates were distributed in seven genera as follows: *Saccharomyces* (23 isolates), *Trichosporon* (13 isolates), *Kluyveromyces* (8 isolates), *Candida* (7 isolates), *Deboryomyces* (7 isolates), *Geotricum* (7 isolates), *Pichia* (3 isolates). According to data obtained all yeasts species, from yoghurt and strained yoghurt samples could be usually considered to be psychrophic because of their ability to grow at refrigeration conditions. Especially the predominance of *S. cerevisiae* in strained yoghurt can be explained by its ability to ferment glucose and galactose and to assimilate glucose, which occurs in appreciable amounts in yoghurt whey as mentioned by Yamani and Abu-Jaber (1994). Also *S. cerevisia* exhibited from other related dairy products, yoghurt and yoghurt starter cultures. The frequent isolations *Kluyveromyces marxianus* important criteria governing the growth of yeasts in yoghurts is the ability to grow well in cold storage and to ferment glucose, galactose and lactose (Table 2). These criteria are consistent with the storage of yoghurt types under refrigerated conditions and the presence of lactose and sucrose as the major carbonhydrates. These findings about the isolations of the lactose fermenting yeast, Kluyveromyces spp, is in agreement with previous studies on yeasts in yoghurt starter culture (Suriyarachi and Fleet, 1981), yoghurt (Spillmann and Geiges, 1983), strained yoghurt (Yamani and Abu-Jaber, 1994), kefir grains (Engel *et al*., 1986).

The remaining yeasts species occurred in this study were previously isolated and identified from different dairy products; *G. candidum*, *T. cutaneum* and *T. brassicae* were isolated from raw milk, quarg and cheese species (Fleet and Mian, 1987; Roostita and Fleet, 1996; Engel *et al*., 1980; Engel, 1986). *C. blankii*, *C. lipolytica*, *C. tropicalis*, *C. kefir* were isolated from pasteurized milk, yoghurt, cheese (Fleet and Mian, 1987; Hatting and Viljoen, 2002). *Debaryomyces hansenii*, *Pichia farinosa*, *P. anemola* were isolated yoghurt, labaneh, butter milk and cheese (Suriyarachi and Fleet, 1981; Yamani, and Abu-Jaber, 1994; Hatting and Viljoen, 2002; Mihyar *et al*., 1997).

Conclusions

The occupance of yeasts in dairy products is not usually uncommon. Mainly psychrotropic yeasts are the main cause of spoliage of traditionaly produced dairy products. Use of alternative producing techniques, instead of the traditional methods, may help to avoid the adverse effects of yeasts growth. The use of yeasts-free starter cultures, application of accurate hygienic measures during processing and packing and maintenance of an effective cold chain from production until consumption could help control spoliage yeasts. On the other hand, the presence of a large number of yeasts during the late stages of ripening suggests that some yeasts species may play an important role in the ripening of medium and long term ripened cheese varieties. Also the occurrence of toxigenic fungi *(Penicillium expansum, P. roquefortii* and *Aspergillus versicolor* etc.) from medium and long term ripened Turkish cheese varieties may be a reason for concern linked to the risk of occurring mycotoxin residues in the end product (Lopez-Diaz *et al*., 1995).

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