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Isolation and Identification of Yeasts from Traditional Yoghurts and Some Microbiological Properties

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Abstract: The aim of the present study was to isolate and identify yeasts from yoghurts produced using traditional methods in the North East Anatolian Region of Turkey. In addition, samples were analysed in terms of certain microbiological characteristics. A total of 96 yeast isolates were obtained from the samples. Distribution of the isolates according to species was 50.00% *Candida kefyr* followed by 23.95% *Saccharomyces cerevisae*, 10.40% *Candida sphaerica*, 7.29% *Candida sake*, 4.16% *Candida lypolitica*, 2.08% *Candida inconspicua*, 1.04% *Candida krusei* and 1.04% *Candida famata*. Coliform bacteria and Enterobacteriaceae counts of the samples were <1.00-3.13 and <1.00-3.22 log cfu g^{-1}, respectively. As mean, total aerobic mesophilic bacteria, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and yeast and mold counts were 7.09±0.92, 5.52±1.08, 7.86±0.94 and 6.32±0.87 log cfu g^{-1}, respectively.

Key words: Yoghurts, sample, *Candida sphaerica*, *Candida famata*, isolate

INTRODUCTION

Homemade yoghurt production has been made in Turkey based on traditional techniques dating back thousands of years (Kurt, 1995; Ozden, 2008). It has been reported that traditional yoghurt production consists of a number of processes in which milk is boiled until it loses approximately 1/3 of its original volume, cooled to body temperature, incubated with a yoghurt sample from the previous day, waited until fermentation ends and later cooled (Yoney, 1967; Ozer, 2006). Industrial yoghurt production based on yoghurt starter cultures besides traditional yoghurt production has been increasing in Turkey (Ozden, 2008). The fermented milks notification defines yoghurt as the product of fermented milk in which symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sub sp. *bulgaricus* are specifically used for fermentation (Anonymous, 2009). According to the Fermented Milks Notification, a minimum count of 10^7 cfu g^{-1} of starter culture bacteria is viable in yoghurt (Anonymous, 2009). According to The Fermented Milks Notification (Anonymous, 2009) and the Standard for yoghurt (Anonymous, 2006), the highest permissible number of coliform bacteria in yoghurt has been determined as 9 MPN g^{-1}, although the maximum count of yeast/mold allowable according to the Fermented Milks Notification (Anonymous, 2009) and The Standard for yoghurt (Anonymous, 2006) as 1×10^2 and 1×10^1 cfu g^{-1}, respectively.

The frequent occurrences of yeast in dairy related products indicate the ability of yeasts to survive and metabolise milk constituents. Yeasts might develop in milk as secondary flora after yogurt culture bacterial growth (Lourens-Hattingh and Viljoen, 2001). It is indicated that yoghurt is a selective environment for yeasts which metabolise galactose occurring by the degradation of lactose and organic acids produced by lactic acid bacteria, in addition to being a low level of pH of yoghurt (Cosentino *et al*., 2001). Although, yeasts have positive activities to produce organic acids, volatile acids, substances that have antibiotic features and many other intermediate products, they are considered to be undesirable microorganisms because they develop in an environment with a low pH level and produce lipolytic and proteolytic enzymes (Viljoen, 2001; Frohlich-Wyder, 2001; Viljoen *et al*., 2003; Mayoral *et al*., 2005). It is indicated that excessive amounts of gas produced by fermentation of yeasts causes swelling of yoghurt containers, loss of flavor and reduction in the texture quality (Mayoral *et al*., 2005).

Yeasts belong to the fungi classes Ascomycetes and Basidiomycetes and they are eukaryotic, heterotrophic and unicellular microorganisms. Yeasts can reproduce sexually by means of sexual spores called ascospores and basidiospore. Yeast that forms either ascospores or basidiospore is referred to as a perfect state. Yeast that does not form either ascospores or basidiospore is referred to as an imperfect state. In current mycological

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term the perfect state is called the teleomorphic state or teleomorph while the imperfect state is called the anamorphic state or anamorph. The teleomorph and anamorph of the same yeast can have different names and synonyms are also used for the same yeast species. *Candida kefyr* is an anamorph of *Kluyveromyces marxianus* and *Kluyveromyces fragilis*, *K. bulgaricus*, *Saccharomyces fragilis* are synonyms of *Kluyveromyces marxianus*. *Kluyveromyces marxianus* var. *marxianus* and *Kluyveromyces marxianus* var. *bulgaricus* are also interchangeably used *Candida kefyr* (Kurtzman and Fell, 2000). *Candida famata* is an anamorph of *Debaryomyces hansenii*. *Torulopsis candida* is a synonym of *Candida famata*. *Candida lipolytica* is ananamorph of *Yarrowia lipolytica*. *Candida sphaerica* is an anamorph of *Kluyveromyces lactis*. *Kluyveromyces marxianus* var. *lactis* are also interchangeably used for *Kluyveromyces lactis*. *Candida krusei* is ananamorph of *Issatchenkia orientalis*. *Candida sake* is a synonym of *Candida tropicalis* (Kurtzman and Fell, 2000).

In a study on the effect of temperature on the development of yeast in yoghurt. It was reported that the most frequently isolated yeast species were *Saccharomyces cerevisiae*, *Debaryomyces hansenii* (*Candida famata*), *Saccharomyces exiguus*, *Kluyveromyces marxianus* (*Candida kefyr*), *Yarrowia lipolytica* (*Candida lipolytica*) and *Rhodotorula glunitis*, respectively (Viljoen *et al*., 2003). It was reported that distribution of the genus in 68 isolates obtained from yoghurt samples were *Saccharomyces* 23 isolate, *Tricosporon* 13 isolate, *Kluyveromyces* 8 isolate, *Candida* 7 isolate, *Debaryomyces* 7 isolate, *Geotricum* 7 isolate and *Pichia* 3 isolate as for the distribution of isolates to species 17 isolates of *Saccharomyces cerevisiae* biovar I, 5 isolates of *Saccharomyces cerevisiae* biovar II, 1 isolate of *Saccharomyces cerevisiae* biovar III, 3 isolates of *Pichiafarinosa*, 1 isolate of *P. anemola*, 1 isolate of *Candida blankii*, 3 isolates of *Candida lipolytica*, 2 isolates of *Candida tropicalis* (*Candida sake*), 5 isolates of *Kluyveromyces marxianus* var. *lactis* (*Candida sphaerica*), 3 isolates of *Kluyveromyces marxianus* var. marxianus (*Candida kefir*), 7 isolates of *Geotricum candidum*, 9 isolates of *Tricosporon cutaneum*, 4 isolates of *Tricosporon brassicae*, 7 isolates of *Debaryomyces hansenii* (*Candida famata*) (Kavas *et al*., 2006). In a survey of retail outlets in Brazil, it was reported that distribution at the level species of 178 isolates obtained from plain yoghurt samples were 48 isolates of *Debaryomyces hansenii* (*Candida famata*), 17 isolates of *Saccharomyces cerevisiae*, 36 isolates of *Hansenula* sp. 40 isolates of *Mrakia frigida*, 7 isolates of *Candida parapsilosis*, 8 isolates of *Debaryomy cescastellii*, 17 isolates of *Candida maltosa*, 3 isolates of *Schizosaccharomyces pombe*, 1 isolate of *Candida mogii* and 1 isolate of *Kluyveromyces marxianus* (*Candida kefyr*) as for distribution at the level species of 409 isolates obtained from fruit yoghurt samples there were 143 isolates of *Debaryomyces hansenii* (*Candida famata*), 97 isolates of *Saccharomyces cerevisiae*, 55 isolates of *Hansenula* sp., 28 isolates of *Mrakia frigida*, 37 isolates of *Candida parapsilosis*, 25 isolates of *Debaryomyces castellii* izolat and 24 isolates of *Candida maltosa* (Moreira *et al*., 2001). In a study carried out on dairy products sold in Austrian retail markets, it was reported that distribution at the level genus of 26 isolates obtained from yoghurt samples were 4 isolates of *Candida* sp., 4 isolates of *Clavispora* sp., 6 isolates of *Debaryomyces* sp., 4 isolates of *Geotrichum* sp., 1 isolate of *Pichia* sp., 1 isolate of *Yarrowia* sp. and 3 isolates of *Rhodotorula* sp. as for distribution at the level species of the isolates were 3 isolates of *Candida pseudoglaebosa*, 1 isolate of *Candida sojae*, 4 isolates of *Clavispora lusitaniae*, 6 isolates of *Debaryomyces hansenii* (*Candida famata*), 4 isolates of *Geotrichum candidum*, 1 isolate of *Pichia guilliermondii*, 1 isolate of *Yarrowia lipolytica* (*Candida lipolytica*) and 3 isolates of *Rhodotorula mucilaginosa* (Lopandic *et al*., 2006). In a study carried out on dairy products produced in different regions of Bulgaria, it was indicated that *Kluyveromyces marxianus* (*Candida kefyr*) was the species of dominant yeastin Yoghurt samples produced from cow's milk, *Trichosporon beigelii*, *Kluyveromyces marxianus* var. *marxianus* (*Candida kefyr*) and *Kluyveromyces marxianus* var. *bulgaricus* (*Candida kefyr*) were 30% and *Debaryomyce shansenii* var. *hansenii* (*Candida famata*), *Candida krusei* and *Candida rugosa* had equal rates (Savova and Nikolova, 2002). In a study carried out on plain and fruit yoghurt samples collected from Australian retail markets, it was reported that distribution at the level genus of 73 isolates obtained from yoghurt samples were 25 isolates of *Torulopsis* sp., 13 isolates of *Kluyveromyces* sp., 13 isolates of *Saccharomyces* sp., 7 isolates of *Candida* sp., 6 isolates of *Rhodotorula* sp., 5 isolates of *Pichia* sp., 2 isolates of *Debaryomyces* sp. (izolat) and 2 isolates of *Sporobolomyces* sp. and *Torulopsis candida* (*Candida famata*) was the most frequently isolated yeast (21 samples) followed by 11 samples of *Kluyveromyces fragilis* (*Candida kefir*), 9 samples of *Saccharomyces cerevisae*, 2 samples of *Kluyveromyces lactis* (*Candida sphaerica*) and 2 samples of *Debaryomyces hansenii* (*Candida famata*) (Suriyarachchi and Fleet, 1981).

MATERIALS AND METHODS

A total of twenty five yoghurt samples (1 kg each) were collected from villages in the Erzurum and Kars regions under aseptic conditions. The samples were transported under normal cold chains to the Department of Food Hygiene and Technology Laboratory, Faculty of Veterinary Medicine, Ataturk University where they were immediately analyzed. The samples were kept in a refrigerator (4±1°C) until the completion of analysis.

Preparation of dilutions: The 25 g of yoghurt samples were homogenised in a stomacher for 2 min in 225 mL of sterile Ringer's solution prepared with ¼ of a ringer tablet. Serial dilutions up to 10^{-7} were carried out using the same homogenate.

Enumeration of yoghurt starter bacteria: *Streptococcus thermophilus* agar (ST agar) (tryptone 10 g, yeast extract 5 g, sucrose 10 g, dipotassium phosphate 2 g, agar 15 g, distilled water 1 L) was used for enumeration of *Streptococcus thermophilus*. Appropriate dilutions were pour-plated. The plates were incubated aerobically at 37°C for 24 h in an incubator. The colonies formed were evaluated by counting them at the end of incubation. The MRS agar at pH 5.2 which had been adjusted with 1 M HCl acid was used for the selective enumeration of *L. delbrueckii* ssp. *bulgaricus*. Appropriate dilutions were pour-plated. The plates in an anaerobic jar were incubated at 45°C for ≥72 h under anaerobic conditions created with the use of Anaerocult (Anaerocult A, Merck) in an incubator. The formed colonies were evaluated at the end of incubation and counted later (Dave and Shah, 1996).

Enumeration of total aerobic mesophilic bacteria: Plate Count Agar (PCA, Merck) was used for enumeration of Total Aerobic Mesophilic Bacteria (TAMB). Appropriate dilutions were pour-plated. The plates were incubated aerobically at 30°C for 72 h. TAMB was counted after the colonies were evaluated (Harrigan and McCance, 1976).

Enumeration of coliform bacteria: Violet Red Bile Agar (VRBA, Merck) was used for enumeration of coliform bacteria. The plates were incubated aerobically at 35±1°C for 48 h after pour plating of the appropriate dilutions. The colonies were evaluated at the end of incubation later the coliform bacteria count was conducted (Harrigan and McCance, 1976).

Enumeration of Enterobacteriaceae: Enterobacteriaceae counts were conducted on Violet Red Bile Dekstrose Agar (VRBDA, Merck). After pour-plated of the appropriate dilutions, each plate was overlaid with 5-8 mL VRBD agar. After the plates were incubated aerobically at 30°C for 48 h, purple red colonies with 0.5 mm diameter were counted (Kornacki and Johnson, 2001).

Enumeration of yeast and mold: Yeast and mold counts were conducted on Rose Bengal Chloramphenicol agar (RBC, Merck). After the pour-plated plates were incubated aerobically for 5 days, the formed colonies were controlled microscopically and counted (Jarvis, 1973). The typical yeast colonies had been used for the isolation of yeast.

Isolation and identification of yeasts: The colonies were classified according to the morphology of the colony. Then, for the enrichment, each of the different yeast colonies which had microscopically be enconsidered as yeast were cultured on RBC agar using the Streak Plate Method and the cultured plates were incubated aerobically at 25°C (Jarvis, 1973). After the enrichment, the uniform colonies (the isolated yeasts) were transferred to fresh agar slants prepared using rose bengal chloramphenicol agar and the isolated yeasts on agar slants was stocked at 4°C in a refrigerator for a mean of 15 days until the process of identification of the isolates.

For identification, the isolated yeasts were re-cultured using the Streak Plate Method to plates which had been prepared with RBC agar, afterwards the plates were incubated at 25°C for 5 days. Finally, the obtained fresh yeast cultures were used for biochemical tests.

The yeast isolates were identified with identification tests with the VITEK 2 System (bioMerieux) according to the instructions of the manufacturer. For this, VITEK 2 yeast cards (VITEK-2 YST, bioMerieux), containing various sugars, enzymes and substrates in their wells as L-lysine-arylamidase, L-malate, Leucine-arylamidase, arginine-GP, erythritol, glycerol, tyrosine-arylamidase, beta-N-acetyl-glucosaminidase, arbutin, amygdalin, D-galactose, gentibiose, D-glucose, lactose, methyl-A-D-glucopyranoside, D-cellobiose, gamma-glutamyl-transferase, D-maltose, D-raffinose, PNP-N-acetyl-BD-galactosaminidase-1, D-mannose, D-melibiose, D-melizitose, L-sorbose, L-rhamnose, xylitol, D-sorbitol, saccharose/sucrose, urease, alpha-Glucosidase, D-turanose, D-trehalose, nitrate, L-arabinose, D-galacturonate, esculin, L-glutamate, D-xylose, DL-lactate, acetate, citrate (sodium), glucuronate, L-proline, 2-keto-D-gluconate, N-acetyl-glucosamine and D-gluconatewere inoculated with the fresh cultures (Pincus, 2006).

RESULTS

Microbiological analysis findings of yoghurt samples: The results were obtained from microbiological examination of the samples. *Streptococcus thermophilus*

Table 1: Some microbiological properties of yogurt samples

Microorganism	Minimum	Maximum	Mean
Streptococcus thermophilus	3.60*	7.08	5.52±1.08
Lactobacillus delbrueckii sp. *bulgaricus*	6.00	9.61	7.86±0.94
TAMB**	5.00	9.43	7.09±0.92
Coliform bacteria	<1	3.13	***
Enterobacteriaceae	<1	3.22	-
Yeast and mold	4.62	7.74	6.32±0.87

*log cfu g^{-1}. **Total aerobic mesophilic bacteria. **It is not calculated

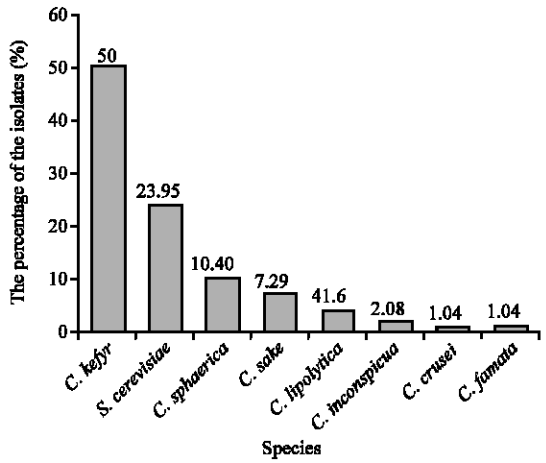

Fig. 1: The percentage of the isolates according to yeast species

counts of the samples ranged between 3.60-7.08 log cfu g^{-1} and had 5.52±1.08 log cfu g^{-1} as the mean. *Lactobacillus delbrueckii* ssp. *bulgaricus* counts of the samples were determined between 6.00-9.61 log cfu g^{-1} and had 7.86±0.94 log cfu g^{-1} as the mean (Table 1). TAMB counts of the samples were between 5.00-9.43 log cfu g^{-1} and were 7.09±0.92 log cfu g^{-1} as the mean, coliform bacteria counts were between <1-3.13 log cfu g^{-1} and Enterobacteriaceae counts were between <1-3.22 log cfu g^{-1} (Table 1). It was determined that yeast and mold counts of the samples varied from 4.62-7.74 log cfu g^{-1} and were 6.32±0.87 log cfu g^{-1} as the mean (Table 1).

Identification of yeasts: The distributions of 96 yeast isolates obtained from the samples at the species level are shown in Fig. 1. It was identified that 73 isolates and 23 isolates belonged to *Candida* sp. and *Saccharomyces* sp. respectively. It was determined that *Candida* sp. and *Saccharomyces* sp. had rates of 73/96 (76.04%) and 23/96 (23.95%) within all isolates, respectively. Conversely, *Candida kefyr*, *Saccharomyces cerevisiae*, *Candida sphaerica*, *Candida sake*, *Candida lipolytica*, *Candida inconspicua*, *Candida crusei* and *Candida famata* had rates of 48/96 (50%), 23/96 (23.95%), 10/96 (10.4%), 7/96 (7.29%), 4/96 (4.16%), 2/96 (2.08%), 1/96 (1.04%) and 1/96 (1.04%) at the species level, respectively (Fig. 1).

DISCUSSION

In this study, it could be said that Coliform bacteria counts of the samples corresponded the rate of 84% to the value specified inthe Turkish Food Codex Fermented Milks Notification (Anonymous, 2009) and the standard of the Turkish Standards Institute for yoghurt (Anonymous, 2006). No yoghurt samples analysed (25/25) in terms of the number of yeasts and molds were consistent with the specified counts inthe fermented milks notification (Anonymous, 2009) and the yoghurt Standard (Anonymous, 2006).

It can be said that the aspect of rate of isolation of *Candida* sp. (73/96) is dominant in the analyzed yoghurts. Similar findings have been reported for the yoghurts produced from cow's milks in different regions of Bulgaria (Savova and Nikolova, 2002). *Candida* sp. were reported as the second most commonly isolated genus in plain and fruit yoghurts consumed in the regions of Izmir and Aydin (Kavas *et al*., 2006) and in yoghurts sold in Austria (Lopandic *et al*., 2006). However, plain and fruit yoghurt samples collected in markets in Australia were reported to have significantly lower genus of *Candida* (Suriyarachchi and Fleet, 1981). It is thought that the results arise from production technique and storage conditions in addition to the difference in materials used in production.

Candida kefyr (anamorph *Kluyveromyces marxianus*) which is the most commonly isolated between yeast species in this study was reported to be the most commonly isolated yeast in cow's yoghurts collected in various regions of Bulgaria (Savova and Nikolova, 2002). Conversely, it was reported that *Candida kefyr* was the least isolated yeast in plain and fruit yoghurts collected in the regions of Izmir and Aydin (Kavas *et al*., 2006) and in plain yoghurt sold in Brazil (Moreira *et al*., 2001). The numbers of isolates determined for *Candida sphaerica* (anamorph *Kluyveromyces lactis*) and *C. sake* (synonym *Candida tropicalis*) are similar to the finding reported by Kavas *et al*. (2006). However, this yeast species could not be determined in some studies carried by Savova and Nikolova (2002), Lopandic *et al*. (2006) and Suriyarachchi and Fleet (1981). *Candida lipolytica* (anamorph *Yarrowia lipolytica*) are less frequently isolated as well asin yoghurts consumed in Austria (Lopandic *et al*., 2006) and in plain and fruit yoghurts sold by Izmir and Aydin (Kavas *et al*., 2006). *Candida krusei* have been detected at a lower rate than the determined rate in yoghurts produced from goat'smilk (Savova and Nikolova, 2002). In this study, *Candida inconspicua* which has been determined in the yoghurt samples, could not be detected in other investigations (Suriyarachchi and Fleet, 1981; Savova and Nikolova, 2002; Kavas *et al*., 2006; Lopandic *et al*., 2006).

Saccharomyces sp. has been reported in many studies (Suriyarachchi and Fleet, 1981; Moreira *et al*., 2001; Kavas *et al*., 2006). As for this study, *Saccharomyces* sp. was determined as the most common second genus in fruit yoghurts sold in Brazil (Moreira *et al*., 2001). However, *Saccharomyces* sp. was detected as the most common genus and *Saccharomyces cerevisiae* was identified as the most common species in the market study which examined plain and fruit yoghurts sold in the regions of Izmir and Aydin (Kavas *et al*., 2006). In the study, however, this yeast species has been determined as the second most common species. *Saccharomyces cerevisiae* was also reported as the second most common species in fruit yoghurts by Moreira *et al*. (2001). Savova and Nikolova (2002) reported that the yeast species had never been identified in yoghurts.

Due to the high number of yeasts, the analyzed samples have shown that yeast contamination could occur in the production method of traditional yoghurt. It could even be said that some yeast species are an essential element of microflorain yoghurts produced by means of traditional production. When the effects of some probiotic beside the adverse effects on yoghurt of yeasts are considered, traditionally produced yoghurts must be considered and evaluated separately from industrially produced yoghurts. In addition, further studies could be done in this regard. In terms of isolated yeast species, yeast species belonging to the genus of *Candida* and *Saccharomyces* were found to be first and second highest, respectively. In all yeast species, *Candida kefyr* with the rate of 50% were identified as the most frequently isolated yeast. Other yeast species belong to the genus of *Candida* is *Candida sphaerica*, *C. sake*, *C. lipolytica*, *C. inconspicua*, *C. krusei* and *C. famata*. In the genus of *Saccharomyces*, *Saccharomyces cerevisiae* have only been isolated from the samples and has been the second most frequently isolated yeast.

CONCLUSION

When microbiological analyses has been considered, it is detected that yoghurts produced using the traditional method in terms of the number of coliform bacteria are appropriate to the standard for yoghurt. In terms of the number of yoghurt culture bacteria, it has been determined that the count of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are low and high, respectively.

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