INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–393/ZII/2010/12–2–231–236
http://www.fspublishers.org

Full Length Article

Comparison of Locally Isolated Culture from Yoghurt (*Dahi*) with Commercial Culture for the Production of Mozzarella Cheese

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ABSTRACT

Mozzarella cheese is getting popularity in Pakistan as the trend of pizza is increasing. In Pakistan very few industries are making mozzarella cheese. Cultures required for the production of mozzarella cheese are imported and expensive, which increase the cost of its production. An attempt was made to develop such cultures locally. Homemade yoghurt (dahi) was used as the source for the culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus.* Cultures were isolated and identified using morphological examination and various biochemical tests and preserved in glycerol at -80°C. These cultures were compared with the commercially available cultures for the production of mozzarella cheese. Locally isolated cheese culture showed higher moisture content, faster acid production and rate of proteolysis in cheese as compared to commercial culture. © 2010 Friends Science Publishers

Key Words: Isolation; Starter culture; Cheese; Chemical composition; TCA soluble nitrogen; Melt time

INTRODUCTION

Mozzarella cheese is the one of the most popular cheese in the world, because of its primary use in the pizza topping (Kindstedt, 2004). The demand of Mozzarella cheeses is also growing in Pakistan as the demand for pizza is increasing. The three basic steps of cheese production, acidification, coagulation and dehydration or syneresis are common in all cheese varieties. After preparation some cheeses are used as fresh, while other are ripened for a period with respect to the variety.

Acidification of milk is basic process in the production of the majority of cheese variety. Accurate rate and time of acidification is the pivotal in processing of good quality cheese (Barbano, 1999; Fox *et al.*, 2000; McSweeney, 2007). To achieve this step food grade acidulates (acetic acid, lactic acid, citric acid) and lactic acid bacterial (LAB) starter cultures are added in the milk prior to processing. A primary function of bacterial culture is to convert the lactose into glucose or galactose, and then these sugars are converted to the end product of lactic acid. The rate of acid production is critical in determining cheese quality. The acidification also contributes to a preservative effect with the result that many pathogenic and spoilage bacteria are inhibited. The associated drop in pH also results in the loss of water from the curd as whey (Banks, 2002). The choice of the starter culture is also important for proper texture and flavor profile (Banks, 2004).

Bacterial starters have been produced for a variety of fermented products to improve their sensory and other quality characteristics (Saeed *et al.,* 2009). Lactic starter culture may consist of single strain used alone or in combination or undefined mixtures of strains (Kosikowski, 1982; Marth & Steele, 2001). Starter cultures are now lyophilized with milk components, nutrients and energizers and distributed commercially in the dry state or these are frozen with liquid nitrogen and distributed (Kosikowski, 1982).

Mozzarella is produced using a paired lactic acid bacteria starter culture comprised of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* or *L. helveticus* (Coppola *et al*., 2001). The close synergistic relationship between *S. thermophilus* and *L. bulgaricus* permits an augmented acid production during milk fermentation. As a result of adaptation to the protein rich milk environment there is increased synthesis of amino acid. *S. thermophilus*, can produce almost all amino acids, but lacks an extra cellular protease. *L. bulgaricus* on the other hand lacks enzyme for synthesizing most amino acids, but it possesses an extracellular caseinolytic protease (Hols *et al.*, 2005).

During culture production, first the bacterial isolates are identified on the basis of morphological and biochemical characteristics. Morphological inspection is

To cite this paper: Sameen, A., F.M. Anjum, N. Huma and M.I. Khan, 2010. Comparison of locally isolated culture from yoghurt (*Dahi*) with commercial culture for the production of mozzarella cheese. *Int. J. Agric. Biol.,* 12: 231–236

carried out based on key characters. Gram positive or negative and catalase positive or negative are the key character to differentiate from LAB (Gerhardt & Murry, 1981; Jay, 2000). The Gram positive and catalase negative cocci are studied for their growth at 10°C and 45°C and in pH 9.6 broths and 4.5-6.5% NaCl broth.

L. bulgaricus is a Gram-positive rod that may appear long and filamentous. It is also non-motile and it does not form spores. It produces D (-) lactate, with a narrow range of fermented carbohydrates, aero-tolerant and has complex nutritional requirements. It requires a relatively low pH (around 5.4-4.6) in order to grow effectively. The organism is found in a variety of habitats, including the gastrointestinal tract and vegetation, or manmade habitats such as sewage and fermenting or spoiling food. Their growth occurs at a temperature of 45ºC and no growth occurs at 15ºC and cell wall peptidoglycan type is Lys-DAsp (Wood & Holzapfel, 1995).

Phenotypic methods like API were commonly used for identification of *Lactobacillus* species. A commercial kit, such as API 50 CHL system, is another method for identification, by analysis of enzymes and sugar-fermentation profiles. A clear identification of isolates on the basis of phenotype criteria alone is often problematic and has poor concordance. Major disadvantages with these methods are that they may give misleading results if applied to unknown bacteria and may not be adequate to reliably distinguish between closely related *Lactobacillus* species (Oust *et al.,* 2004).

In Pakistan, all the cultures for fermented dairy products like yoghurt and cheese are imported in the form of liquid, spray dried, and freeze dried and frozen forms (Zahoor *et al.,* 2003), which are very expensive for small and large scale cheese producers. The culture manufacturing companies mainly entertain bulk orders, so small scale cheese manufacturers are sometime in problems. Considering these issues there is dire need to produce these cultures in Pakistan to reduce the cost of production and ensure the availability of cultures for the small scale cheese producers. Hence this study was carried to develop the Mozzarella cheese cultures in deep frozen form and to find the efficacy of cultures in the manufacturing of Mozzarella cheese.

MATERIALS AND METHODS

Development of indigenous cultures: Ten different samples of homemade yoghurt (*dahi*) procured from local market were used as source for isolation of indigenous culture. All the curd samples were diluted at a ratio of 1: 10 in distilled water, transferred to sterilized screw capped test tubes and stored at 4°C. Twenty petri plates, containing nutrient agar were prepared and one mL of each dilution was poured separately on plates containing solid agar medium and then spread with a sterile rod. The petri plates were incubated at 37°C for 48 h.

Morphological study: Morphological characteristics of each colony appearing on Nutrient agar were studied by preparing glass slides aseptically as described by Cappuccino and Sherman (1996). Heat fixation was done by passing the glass slide for two to three consecutive movements over the flame of burner. During heat fixation bacterial proteins coagulated and fixed to glass surface. For Gram's staining, 24 h old agar plate culture was used as described by Harrigan and McCanc (1976).

Purification and identification of cultures: Morphological examined colonies of *S. thermophilus* and *L. bulgaricus* with desired characters were shifted onto the surface of deMan, Rogosa and Sharpe (MRS) and β–disodium glycerophosphate (M17) agar plates. *L. bulgaricus* and *S. thermophilus* were then transferred into MRS broth M17 broth respectively and incubated at 37ºC and preserved in refrigerator at 4ºC for further use. The results of colony characteristics were recorded. For confirmation and characterization of bacterial cultures, sugar fermentation, biochemical (methyl red test, Vogas-proskauer test, indole production, citrate utilization & litmus milk reaction) enzyme activity tests (hydrogen sulphide production, catalase, oxidase, urease & starch hydrolysis) were carried out according to the methods described by Cappuccino and Sherman (1996). The purified and identified bacterial strains of *S. thermophilus* and *L. bulgaricus* were preserved in glycerol at -80°C for use in cheese manufacturing.

Procurement of raw materials: Buffalo raw milk was procured from Dairy Farm, University of Agriculture, Faisalabad, Pakistan, for the preparation of Mozzarella cheese. Commercially available Mozzarella cheese culture (*S. thermophilus* & *L. delbrueckii subsp bulgaricus*) from Chr. Hansen's Laboratory (Ireland) Ltd, Cork, Ireland was also used for cheese manufacturing for comparison with locally isolated culture. The enzyme chymosin (Double strength Chy-max, 500000 MCU/mL, Pfizer Inc, Milwaukee, WI, USA) was used to coagulate the milk in the present study.

Cheese preparation: The buffalo milk used for cheese production was first standardized at 0.9 casein fat ratio and then pasteurized at a temperature of 65°C for 30 min. After this the milks were divided into two parts and cooled to 37°C. One part of milk was inoculated with indigenous cultures isolated and preserved in the Lab @ 2% (*L. bulgaricus* & *S. thermophilus* 2:1 ratio, respectively) and the other part with commercial culture (@ 2%). After 30 min of ripening at 37°C, curd was set with Chymosin @ 0.077 mL/kg of milk. Approximately 40 min after chymosin addition, the curd was cut and then allowed to heal for 10 min. After healing, the periodic gentle agitation of curd to prevent matting. The temperature was increased gradually to 42°C during stirring. The whey was drained at pH 6.2 and curd was matted, followed by cutting of matted curd into pieces and turned every 15-20 min and milled at pH 5.2. The dry

salting was done at the rate of 1.5% of the curd weight. The salted curd was then hand stretched in hot water (70°C) until the uniform and elastic cheese consistency was achieved. The curd was then molded and vacuums packed and stored at 4°C for ripening.

Composition of cheese: Cheese was analyzed for moisture, fat, protein, lactose pH and acidity, (AOAC, 1990). Lactic acids content was determined with high performance liquid chromatography (HPLC) as described by Akalin *et al.* (2002). For determination of TCA soluble nitrogen 1.5 g cheese sample was grinded and mixed with 20 mL of 12% TCA solution followed by filtration through Whatman No.42 filter paper. The measurement was done by Kjeldhal digestion and distillation method of David *et al.* (2004).

Melt time: Melt time of shredded cheese to melt and fuse into a molten mass free of shred identity was determined as described by Guinee *et al.* (2000).

Statistical analysis: Results obtained from different parameters were subjected to statistical analysis using Analysis of Variance Technique (ANOVA) under completely randomized designs (CRD) as described by Steel *et al.* (1997) to evaluate the influence of starter culture on quality and acceptability of Mozzarella cheese using minitab V 11.1. Duncon's multiple range (DMR) test was applied to find the difference between means.

RESULTS AND DISCUSSION

After inoculation of curd samples onto the surface of nutrient agar plates, in most cases more than one type of colonies were noted. Majority of the colonies were of rods and cocci shaped bacteria (Table I). From the total samples 40% rods, 35%, cocci and 25% cluster were observed on nutrient agar plates as reported previously by Cappuccino and Sherman (1996) and Harrigan and McCance (1976).

Purification of isolates of *L. bulgaricus* and *S. thermophilus*: The purification of cultures of *L. bulgaricus* revealed single type of bacterial colonies after 48 h incubation at 37ºC from both curd samples. The colonies on MRS agar plates were 1.5-2.0 mm in diameter, convex, entire and opaque and without pigment (Table II), as reported by John *et al.* (1994). For *S. thermophilus* during purification the colonies of single type were appeared on M17 plates after 24 to 48 h of incubation (Table II). The colonies on M 17 plates were small in size, white in colour, pin point lens shaped, smooth and 0.5-1.2 µm in diameter. The bacteria were G+ve cocci when stained with Gram staining. These results are matched with the findings of Tzanetakis *et al.* (2001). The M17 medium was found to be one of the best selective medium for the isolation and purification of S. *thermophilus* and 100% growth of *S. thermophilus* were observed as described by Mora *et al.* (2005).

Sugar fermentation tests: The bacterial culture S_1 and S_4, were found homofermentative in sugar fermentation test (Table III). In case of lactose and glucose no gas was produced, whereas sucrose, fructose, galactose, inulin, glycerol, maltose and sorbitol were found negative for both acid and gas production, which confirmed the isolated pure culture of *L. bulgaricus* (Erdogrul & Erbilir, 2006). Likewise cultures S_2 and S_3 were also homofermentative. The bacterial culture fermented only

Table I: Cultural and morphological characteristics of bacteria isolated from curd on nutrient agar medium

Samples	Nutrient Agar Plate	Morphological Characteristics
S1	White, regular, pinpoint	G+ve short rods G+ve cocci
S2	White, small, regular, white pinpoint	G+ve short rods G+ve cocci
S3	White regular, white pinpoint	G+ve short rods G+ve cocci
S4	White regular, White irregular	G+ve long rods G+ve short rods
S5	Yellow regular, White regular	G+ve clusters G+ve rods
S6	White, irregular, white pinpoint	G+ve rods
S7	Yellow regular & regular pinpoint	G+ve clusters G+ve cocci
S8	Regular white pinpoint & white irregular	G+ve cocci G+ve short rods
S9	Small white small yellow regular	G+ve rods & cooci G+ve clusters
S10	Yellow pinpoint and yellow regular	G+ve clusters G+ve cocci

Fig. 1: Effect of starter culture on the TCA soluble nitrogen of Mozzarella cheese during ripening
IC= Indigenous culture CC= Commercial culture

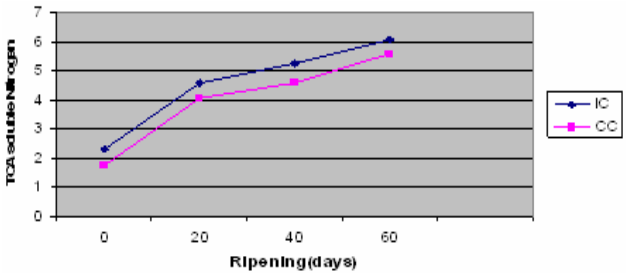

Fig. 2: Effect of starter culture on the melt time of Mozzarella cheese during ripening
IC= Indigenous culture CC= Commercial culture

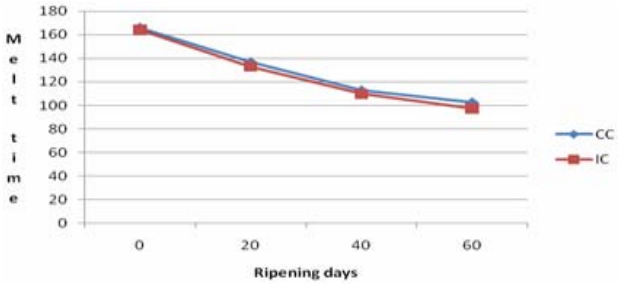

Table II: Growth characteristics on selective media

Samples	MRS Agar plate	Morphological Characters	MRS broth	Morphological Characters
S_1	Opaque, white, convex, regular	G+ve rods short chain	Turbidity & sedimentation	Gram+ve short rods
S_4	Opaque, long, convex and entire	G+ve rods short & long chain	Turbidity & some sedimentation at bottom	Gram+ve short & long chains
S_6	White regualar & irregular White pinpoint M17 Agar plate	G+ve cocci & G+ve short rods	Clear, some sedimentation M17 broth	Gram+ve cocci Gram+ve rods
S_2	White, small, smooth and pinpoint	G+ve cocci in short chain	Turbidity & growth	Gram+ve short chain & spherical cocci
S_3	White lense shaped, pinpoint, white regular	G+ve cocci in pairs & chain	Turbidity & sedimentation	Gram+ve cocci in pairs & short chain
S_8	White irregular long & White pinpoint	G+ve rods & G+ve cocci	No turbidity	Gram+ve small and long rods

lactose, sucrose, fructose and glucose without the production of gas. Whereas no acid as well and gas production occurred in case of maltose, sorbitol, inulin, mannitol, raffinose and galactose (Table III). These results verified the purity of *S. thermophilus* (John *et al.,* 1994).

Biochemical and enzyme activity tests: The biochemical tests raveled that the Vogas-proskauer, methyl red test and litmus milk test was +ve for S_1 and S_4. These two strains were –ve for citrate utilization test and indole production test (Table IV). The findings of Marth and Steele (2001) showed that *S. thermophilus* does not utilized citrate and the results of John *et al.* (1994) verify that *S. thermophilus* is negative for indole production test, which also confirmed the isolated pure culture of *S. thermophilus.* The methyl red test was also +ve for the S_2 and S_3 but the other entire biochemical test were –ve for S2 and S3. The results are similar to the finding of (Holt *et al.,* 1994) who stated that *L. bulgaricus* produces red color in the medium.

The enzyme activity tests such as H_2S production, catalase, oxidase, urease activity and starch hydrolysis tests were –ve for all the strain S_1 S_4 S_2 and S_3 except starch hydrolysis test, which was variable for S_2 (Erdogrul & Erbilir, 2006). Barbano and Hitchkiss (2001) also reported that *S. thermophilus* does not produced H_2S. Similarly the bacterial cultures did not show any bubbling (Table IV), which corroborated the findings of Barbano and Hatchkiss (2001) and John *et al*. (1994) and Wood and Holzapfel (1995), signifying that the *S. thermophilus* is catalase negative. The bacterial culture was found negative for oxidase test because it did not develop dark red, purple and black color (Table IV), as reported by Erdogrul and Erbilir (2006).

Cheese composition: The starter culture significantly influenced the moisture, protein, acidity and pH and lactose (Table V). Indigenous culture cheese contained greater moisture (48.40%), acidity (0.97%) and low protein (28.07%), lactose (0.09%) and pH (5.19%) than the cheese from commercial culture, which showed 47.85%, 28.39%, 0.97%, 5.25% values for moisture, protein, acidity and pH, respectively. The ash content of cheese remained unaffected due to difference in culture. The variation in moisture may be related to the faster acid production rate in the indigenous cultured cheese as compared with the commercial cheese, which had lesser moisture content (Dave *et al.,* 2003a). The lactose is converted into lactic acid during cheese making by the starter culture (Azarnia *et al.*, 2006) therefore lactic acid is the most abundant organic acid in all type of cheese (Izco *et al.*, 2002). The lactic acid content of mozzarella cheese prepared from indigenous culture was significantly higher than commercial source.

Results presented in Fig. 1 indicates that the cheese prepared using indigenous culture had higher content of 12% TCA soluble nitrogen than the commercial cultured cheese, although an increase in this character was evident in both the cheeses. This difference was due to the wide variation in the proteolytic activity of the different starter culture at strain level (Oberg *et al.,* 1991). The results further showed that starter culture also showed significant

Table III: Sugar fermentation test for different strains

Sugars	S_1	S_4	S_2	S_3
Lactose	+ve	+ve	+ve	+ve
Sucrose	-ve	-ve	+ve	+ve
Fructose	-ve	-ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve
Galactose	-ve	-ve	+/-ve	-ve
Manitol	-ve	-ve	-ve	-ve
Sorbitol	-ve	-ve	-ve	-ve
Inulin	-ve	-ve	-ve	-ve
Maltose	-ve	-ve	-ve	-ve
Glycol	-ve	-ve	-ve	-ve
Raffinose	-ve	-ve	+/-	-ve
Trechalose	-ve	-ve	-ve	-ve

+ = Acid production, gas production - = No acid production, No gas production +/- = Variable

Table IV: Biochemical and enzymatic test for different selective strains

Biochemical test	S_1	S_4	S_2	S_3
Vogas-proskauer test	+ve	+ve	-ve	-ve
Methyl red test	+ve	+ve	+ve	+ve
Indol production test	-ve	-ve	-ve	-ve
Citrate Utilization test	-ve	-ve	-ve	-ve
Litmus milk test	+ve	+ve	-ve	-ve
Enzyme test				
H_2S production test	-ve	-ve	-ve	-ve
Catalase test	-ve	-ve	-ve	-ve
Oxidase test	-ve	-ve	-ve	-ve
Urease activity test	-ve	-ve	-ve	-ve
Starch hydrolysis test	-ve	-ve	+/-	-ve

Table V: Chemical composition (%) of cheeses prepared from indigenous culture (IC) and commercial culture (CC)

Cheeses	Moisture (%)	Protein (%)	Fat (%)	Acidity (%)	pH	Ash (%)	Lactose (%)	Lactic acid (ppm)	Melt time (sec)
IC	48.40a	28.07b	17.47a	0.97a	5.19b	3.26a	0.09b	12995.6a	126.1b
CC	47.85b	28.39a	17.56a	0.92b	5.25a	3.22a	0.13a	12558.3b	129.5a

Mean carrying same letters are statistically non-significant

effect on the melting time of different Mozzarella cheese. The melting time for indigenous cultured cheese was found 126 sec and for commercial cultured cheese it was 129 sec (Fig. 2). The effect of starter culture on melt time of cheese was also reported by Dave *et al.* (2003b) when they used single culture (*S. thermophilus*) and mix culture (*S. thermophilus & L. helveticus),* which was longer for cheese prepared with mixed culture. The significance difference among the cultures in the present study could be due to the minor difference in their rate of proteolysis of the cultures. The indigenous culture showed more proteolysis as compared to commercial culture in the present study as shown by the higher value of 12% TCA soluble nitrogen.

CONCLUSION

Indigenous culture cheese has higher moisture content and faster acid production rate as compare to commercial culture. The rate of proteolysis was also higher in cheese prepared from indigenous culture as indicated by TCA soluble nitrogen. Indigenous resources for value addition of local product especially in the dairy sector could be worthwhile. Result suggests a strong need to explore local sources for the nourishment of cheese industry.

REFERENCES

Akalin, A.S., S. Gonc and Y. Akbas, 2002. Variation in organic acids content during ripening of pickled white cheese. *J. Dairy Sci*., 85: 1670–1676

AOAC, 1990. *Official Methods of Analysis,* 15th edition. The Association of Official Analytical Chemists, Arlington, Virginia

Azarnia, S., N. Robert and B. Lee, 2006. Biotechnological methods to accelerate Cheddar cheese ripening. *Crit. Rev. Biotechnol*., 26: 121–143

Banks, J.M., 2002. *Cheddar-Type Cheese*. Hannah Research Institute, Ayr, Elsevier, Science Publication, UK

Banks, J.M., 2004. The technology of low fat cheese manufacturing. *Int. J. Dairy Technol*., 57: 199–207

Barbano, D.M., 1999. Controlling functionality of Mozzarella cheese through process control. *Proc. Marschall Italian Cheese Sem., Rhone-Poulenc*, Madison, Wisconsin

Barbano, D.M. and J.H. Hotchkiss, 2001. Characterization and classification of *Streptococcus thermophilus* isolated from yogurt. *J. Dairy Sci*., 85: 1023–1030

Cappuccino, J.G. and N. Sherman, 1996. *Microbiology: A Laboratory Manual*. The Benjamin/Cummings, Menlo Park, California

Coppola, S., G. Blaiotta, D. Ercolini and G. Moschetti, 2001. Molecular evaluation of microbial diversity occuring in different types of mozzarella cheese. *J. Appl. Microbiol*., 90: 414–420

Dave, I.R., P. Sharma and D.J. McMahon, 2003a. Melt and rheological properties of mozzarella cheese as affected by starter culture and coagulating enzymes. *Lait*, 83: 61–77

Dave, I.R., P. Sharma and K. Muthukumarappan, 2003b. Effect of starter culture and coagulating enzymes on viscoelastic behavior and melt of Mozzarella cheese. *J. Food Sci*., 68: 1404–1410

David, W.E., K.R. Michelle, W.H. Malcolm and R. Peter, 2004. Salt induced structural changes in mozzarella cheese and impact upon free oil formation in ripening cheese. *Lait*, 84: 539–549

Erdogrul, O. and F. Erbilir, 2006. Isolation and characterization of *Lactobacillus bulgaricus* and *Lactobacillus casei* from various food. *Turkish J. Biol*., 30: 39–44

Fox, P.F., T.P. Guinee, T.M. Cogan and P.L.H. McSweeney, 2000. *Fundamentals of Cheese Science*. Aspen publishers Inc., Gaithersburg, Maryland

Gerhardt, P. and R.G.E. Murray, 1981. *Manual of Methods for General Bacteriology*. American Society of Microbiology, Washington, DC

Guinee, T.P., M.A.E. Aut and M.A. Fenelon, 2000. The effect of fat on heat rheology, microstructure and heat induced functional characteristics of cheddar cheese. *Int. Dairy J*., 10: 277–288

Harrigan, W.F. and M.E. McCance, 1976. *Laboratory Methods in Food and Dairy Microbiology.* Academic Press, London

Hols, P., F. Hancy and L. Fontaine, 2005. New sights in the molecular biology and physiology of *Streptococcus thermophillus* revealed by comparative genomics. FEMS *Microbiol. Rev.,* 29: 435–463

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. *Bergey 's Manual of Determinative Bacteriology*, 9th edition. Williams and Wilkins, 428-East Preston Street, Baltimore, Maryland

Izco, J.M., M. Tormo and R. Jimenez-Flores, 2002. Rapid simultaneous determination of organic acids, free amino acids and lactose in cheese by capillary electrophoresis. *J. Dairy Sci*., 85: 2122–2129

Jay, J.M., 2000. Fermentation and fermented dairy products. *In*: *Modern Food Microbiology*, 6th edition, pp: 113–130. An Aspen Publication, Aspen Publishers, Inc. Gaithersburg, Maryland

John, G.H., R.K. Neol, H.A.S. Peter, T.S. James and T.W. Stanely, 1994. *Bergey 's manual of Determinative Bacteriology*, p: 61. Williams and Welkins, Maryland

Kindsted, P., M. Caric and S. Milanovic, 2004. Pasta-filata cheeses. *In*: *Cheese Chemistry, Physics and Microbiology*, Vol. II, 3rd edition, pp: 251–277. Elsevier Academic Press, Amsterdam

Kosikowski, F., 1982. *Cheese and fermented milk foods*, pp: 16–17. F.V. Kosikowski and Associates, New York

McSweeney, P.L.H., 2007. *Cheese Problems Solved*, p: 18. Woodhead Publishing Limited, Cambridge, England

Marth, E. and J. Steele, 2001. Starter cultures and their use. *In*: *Applied Dairy Microbiology*, 3rd edition, pp: 131–173. Mackerel Dekker Inc., New York

Mora, D., M.G. Fortina, C. Parini, G. Ricci, M. Gatti and G. Giraffa, 2005. Genetic diversity and technological properties of *Streptococcus thermophillus* strains isolated from dairy products. *J. Appl. Microbiol.,* 93: 278–287

Oberg, C.J., A. Wang, L.V. Moyes, R.J. Brown and G.H. Richard, 1991. Effect of proteolytic activity of thermophilic starter culture on physical properties of Mozzarella cheese. *J. Dairy Sci.,* 74: 389–397

Oust, A., T. Moretro, C. Kirschner and A. Kohler, 2004. FT-IR spectroscopy for identification of closely related lactobacilli. *J. Microbiol. Methods*., 59: 149–162

Saeed, M., F.M. Anjum, T. Zahoor, H. Nawaz and S.U. Rehman, 2009. Isolation and characterization of starter culture from spontaneous fermentation of sourdough. *Int. J. Agric. Biol.*, 11: 329–332

Tzanetakis, N., D. Petridis and V. Xanthopoulos, 2001. Characterization and classification of *S. thermophillus* and *L. bulgaricus* strains isolated from traditional Greek yoghurt. *J. Food Sci*., 66: 747–752

William, M. and S. Wilkins, 1994. *Bergey's Manual of Determinative Bacteriology*, 9th edition. First Preston Street Baltimore, Maryland

Wood, B.J.B. and W.H. Holzapfel, 1995. *The Genera of Lactic acid Bacteria*, 1st edition. Printed in Great Britain by Edmunsbury Press, Bury St. Edmunds, Glassgow, New Zealand

Zahoor, T., S.U. Rehman and U. Farooq, 2003. Viability of *L. bulgaricus* as Yoghurt culture under different preservation methods. *Int. J. Agric. Biol.,* 5: 46–48

(Received 07 November 2009; Accepted 12 December 2009)