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Assessment of Physiological Properties of Some Lactic Acid Bacteria Isolated from Traditional Iranian Dairy Products - Iranian Local Lactic Acid Bacteria Properties

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The isolation and identification of natural starters is a need not only for the dairy industry, which still import starters abroad, but also for the preservation of natural lactic acid bacteria diversity of Iran. With this perspective, the aim of our study was the isolation of starters from local Iranian yoghurts and doogh, and studying about their biochemical characterization. Biochemical identification such as gas production from glucose and citrate, sugar fermentation, ammonia production from arginine, growing temperature and heat stability were down for strain identification. In this study, 23 cocci and 47 bacilli were isolated from local yoghurt and doogh. we found that the most of isolates from local dairy products are lactobacillus,homofermentative with the typical sugar fermentation profile and best growing temperature in 42°C.

Key words: Doogh, Homofermentative, Starter, Yoghurt.

Lactic acid bacteria, found widely in nature and milk products, these bacteria are used as starter cultures in fermented products such as yoghurt, kefir, and, etc. For the better organoleptic properties, extended shelf life, easier digestibility and the specific structure of fermented dairy products, there is a great interest to consumption and production of these types of milk products in some countries[1-3].

The isolation and identification of natural starters is a need not only for the dairy industry, but also for the preservation of natural lactic acid bacteria diversity of one particular area[1,4,5].

In Iran, a variety of dairy products are with the origin of goat's milk, sheep and cattle and, etc. was produced for centuries, such as yoghurt, types of cheese Kashk and Ghara-ghooroot Some of these products not only are specific for Iran but also unique flavor, odor and texture were characterized only in specific areas of this country that due to local lactic acid bacteria properties[1, 6].

With this perspective, the aim of our study was the isolation of lactic acid bacteria from local Iranian yoghurts and doog, and study about their biochemical properties for using as an Iranian yoghurt starter culture to create new organoleptic results.

MATERIALS AND METHODS

Yoghurt sample collection

During to spring 2013, traditional yoghurt samples were collected from northern and northeastern area of Iran, that we sure about not

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to be present of commercial strains there. Samples were collected under aseptic conditions and sampling was done according to ISO 707. The collected samples under hygienic and cool condition was transferred to lab in short time[7, 8].

Lactic acid bacteria isolation

For isolation of lactic acid bacteria, according to International microbiology standard (standard, 2003: standard 2006), samples were homogenized and weighted from each traditional collected yoghurt sample, a 1:10 dilution was made by using peptone water followed by making a 10 fold of serial dilution. 0.1 ml from each dilution was then sub cultured in duplicate in to the MRS agar (Merck, Germany) used for isolation local lactobacilli and M17 agar used for local streptococci isolation[2, 9-12].

MRS cultures were incubated at the appropriate temperature 37°C for 48-72h in anaerobically CO2 incubator and M17 cultures were incubated at 42°C in normal aerobic incubator for 48-72h[13]. Colonies were selected according to the shape and size and streak plating was then used to purify the local strains and kept in MRS and M17 broth with 50% sterile glycerol (Merck , Germany) in -70°C for long term storage of strains in further use[4, 10].

Identification and characterization

In the beginning, all of the strains colonies on MRS and M17 agar were isolated and examined about gram staining and catalase test and microscopic morphology. Gram positive and catalase negative strains were selected for another analysis at the later stage[11, 14].

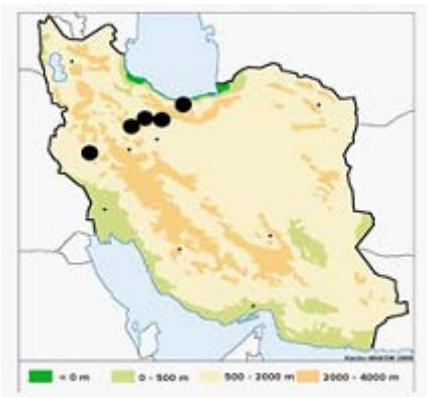

Fig. 1. Sampling area in Iran and the detection of yoghurt and doogh local starter culture

Table 1. Sampling area and samples specifications

Code of samples	Location	Source	pH	LB	ST	Total	cfu of MRS	cfu of M17	Ratio
SA1	Sarab	Yoghurt	3.86	4 (LB1-LB4)	2(ST1-ST2)	6	1.27E+10	6.00E+09	0.47
SA2	Sarab	Yoghurt	3.91	7(LB5-LB11)	3(ST3-ST5)	10	1.40E+10	8.30E+09	0.59
T1	Takestan	Yoghurt	3.96	7(LB12-LB18)	1(ST6)	8	1.51E+10	1.90E+09	0.12
T2	Takestan	Yoghurt	4.03	2(LB19-LB20)	1(ST7)	3	1.16E+10	6.30E+09	0.54
SB	Siah Bisheh	Yoghurt	3.82	2(LB21-LB22)	2(ST8-ST9)	4	1.47E+10	1.51E+10	1.03
J1	Javaher Deh	Yoghurt	3.97	3(LB23-LB25)	1(ST10)	4	1.32E+10	5.80E+09	0.43
TA1	Taleqan	Doogh	3.82	2(LB26-LB27)	4(ST11-ST14)	6	1.17E+09	3.20E+09	2.74
TO1	Tonekabon	Doogh	3.86	6(LB28-LB33)	3(ST15-ST17)	9	1.38E+10	7.80E+09	0.56
TO2	Tonekabon	Yoghurt	3.91	8(LB34-LB41)	4(ST18-ST21)	12	1.41E+10	7.20E+09	0.51
H1	Hamedan	Doogh	3.81	6(LB42-LB47)	2(ST22-ST23)	8	1.12E+10	3.80E+09	0.33

Gas production from glucose

50µl of overnight activated cultures were inoculated in 8ml MRS/M17 containing inverted Dourham tube and accumulative gas in tube was observed after 5 days incubation at 37°C and 42°C[8, 15].

Growth temperature

50µl activated overnight culture was inoculated to 5ml MRS/M17 broth and the turbidity determination according to an optical density at 620µm after incubation in 10, 15, 37, 42 ,45 and 48°C for 7 days[15, 16].

Heat survival tests

A heat survival evaluation was performed at 60 and 65°C for 30, 60 and 90 minutes. For this purpose , 10ml MRS ad M17 broth was prepared in test tube and heated until 60 and 65°C in a water-bath. After that cooled immediately , and inoculated by standard inoculates of each strain and holding in 60 and 65°C for 30, 60 and 90 minute.over heating incubated at 37°C[16].

The optical density at 620 nm was used for analysis cell growth after 7 days incubation in comparison to control sample in the same condition without any heating[16].

Gas production from citrate

All of the heterofermantative lactic acid bacteria have grown well in the presence of citrate and some of them produce gas from citrate.Gas production from citrate was evaluated by the method described by Gibson and Abed-el-Malek in 1945[17].

Ammonia production from arginine

Ammonia production from arginine in streptococci was assessed by using tomato glucose broth as a basal medium with 0.8% (w/v) L-arginine hydrochloride, added before autoclaving. After 14 days incubation, greenish color in the media immediately after adding Nessler's solution indicates arginine hydrolysis and ammonia production by streptococci[16].

Sugar fermentation

Isolates were analyzed for their ability to ferment 6 different sugar (glucose, lactose, sucrose, fructose, galactose and manose) according to turbidity determination of growth media with 2% of sterilized sugar at 620 nm[15].

Statistical analysis

One way analysis of variance (ANOVA) tests were performed in order to perform comparisons between groups. Statistical significance was declared at $p \leq 0.05$ and analysis were performed with prism 6.

RESULTS AND DISCUSSIONS

10 yoghurt and doogh samples were collected from six regions of Iran that were shown in fig. 1. By(•) as collecting area.

In total 130 bacteria were isolated from both M17 and MRS plates and 70 isolates selected after isolation and purification that 47 of them were MRS isolates and 23 of them were M17 isolated.

The origin of samples, source of bacteria the number of *lactobacillus bulgaricus* ,*streptococcus thermophilus* and total number of isolates, pH of each sample,colony forming unit (cfu) in MRS and M17 plate and the ratio between cfu on M17/MRSare shown in table 1.

The means of MRS counts were found to be higher than the viable counts of M17. The expected M17/MRS ratio according to tamime is between 1-10, and in this research except for the SB and TA1, other samples were lower than the expected value[3].The amounts of lactic acid bacteria for a normal yoghurt fermentation should be between 10^8-10^9 for both of species.The amount of colony counts when were compared to these value seemed to be higher than optimum amounts[18] (Table 1).

Biochemical identification

All of 70 isolates subjected to a gram staining and morphology were examined under light microscope. According to blue purple color with gram stain, all of them were gram positive bacteria. The MRS isolated were all bacilli(LB1-LB47) with long and rounded ends, and the isolates coming from M17 were all cocci(ST1-ST23) with spherical or ovoid morphology in chain forming (Table 1.) None of the isolates showed catalase activity in the catalase activity test (Table 2).

The isolates were also subjected to the test for gas production from glucose and in except ST16,ST19 ,ST20 and ST22 were observed in Durham tubes, and this result confirmed the homofermentative behavior of the isolates, while ST16,ST19 ,ST20 and ST22 isolated which isolated from doogh showed heterofermentative behavior with gas production in Durham tubes(Table 2,3,4).

Another test for identification was the

Table 2. Summary of biochemical and physiological properties of isolates

Isolate code	Gram	Catalase	Gas production from glucose	Grow at 15ºC	Grow at 45ºC	Best growing T ºC
LB1	+	-	-	-	+	37
LB2	+	-	-	-	+	42
LB3	+	-	-	-	+	42
LB4	+	-	-	-	+	42
LB5	+	-	-	-	+	37
LB6	+	-	-	-	+	42
LB7	+	-	-	-	+	42
LB8	+	-	-	-	+	42
LB9	+	-	-	-		42
LB10	+	-	-	-	+	37
LB11	+	-	-	-	+	42
LB12	+	-	-	-	+	42
LB13	+	-	-	-	+	42
LB14	+	-	-	-	+	42
LB15	+	-	-	-	+	37
LB16	+	-	-	-	+	37
LB17	+	-	-	-	+	37
LB18	+	-	-	-	+	42
LB19	+	-	-	-	+	42
LB20	+	-	-	-	+	42
LB21	+	-	-	-	+	42
LB22	+	-	-	-	+	42
LB23	+	-	-	-	+	42
LB24	+	-	-	-	+	42
LB25	+	-	-	-	+	42

Table 3. Summary of biochemical and physiological properties of isolates

Isolate code	Gram	Catalase	Gas production from glucose	Grow at 15ºC	Grow at 45ºC	Best growing T ºC
LB26	+	-	-	-	+	42
LB27	+	-	-	-	+	37
LB28	+	-	-	-	+	37
LB29	+	-	-	-	+	37
LB30	+	-	-	-	+	42
LB31	+	-	-	-	+	42
LB32	+	-	-	-	+	42
LB33	+	-	-	-	+	42
LB34	+	-	-	-		37
LB35	+	-	-	-	+	37
LB36	+	-	-	-	+	42
LB37	+	-	-	-	+	42
LB38	+	-	-	-	+	37
LB39	+	-	-	-	+	37
LB40	+	-	-	-	+	42
LB41	+	-	-	-	+	42
LB42	+	-	-	-	+	37
LB43	+	-	-	-	+	37
LB44	+	-	-	-	+	48
LB45	+	-	-	-	+	37
LB46	+	-	-	-	+	42
LB47	+	-	-	-	+	42

Table 4. Summary of biochemical and physiological properties of isolates.

Isolate code	Gram	Catalase	Gas production from glucose	Grow at 10ºC	Grow at 45ºC	Best growing T ºC
ST1	+	-	-	-	+	42
ST2	+	-	-	-	+	48
ST3	+	-	-	-	+	42
ST4	+	-	-	-	+	42
ST5	+	-	-	-	+	42
ST6	+	-	-	-	+	42
ST7	+	-	-	-	+	42
ST8	+	-	-	-	+	42
ST9	+	-	-	-		37
ST10	+	-	-	-	+	42
ST11	+	-	-	-	+	42
ST12	+	-	-	-	+	42
ST13	+	-	-	-	+	42
ST14	+	-	-	-	+	48
ST15	+	-	-	-	+	48
ST16	+	-	+	-	+	42
ST17	+	-	-	-	+	42
ST18	+	-	-	-	+	42
ST19	+	-	+	-	+	42
ST20	+	-	+	-	+	42
ST21	+	-	-	-	+	42
ST22	+	-	+	-	+	42
ST23	+	-	-	-	+	37

Table 5. Summary of biochemical and physiological properties of isolates.

Isolate code	Heat survival at 60ºC			Heat survival at 65ºC			Gas from citrate	Ammonia from arginine
	30min	60min	90min	30min	60min	90min	-	-
LB1	-	-	-	-	-	-	-	-
LB2	-	-	-	-	-	-	-	-
LB3	-	-	-	-	-	-	-	-
LB4	+	-	-	-	-	-	-	-
LB5	+	-	-	-	-	-	-	-
LB6	+	-	-	-	-	-	-	-
LB7	-	-	-	-	-	-	-	-
LB8	-	-	-	-	-	-	-	-
LB9	-	-	-	-	-	-	-	-
LB10	+	+	-	+	-	-	-	-
LB11	+	+	-	+	-	-	-	-
LB12	+	-	-	-	-	-	-	-
LB13	-	-	-	-	-	-	-	-
LB14	+	-	-	-	-	-	-	-
LB15	+	+	-	-	-	-	-	-
LB16	-	-	-	-	-	-	-	-
LB17	-	-	-	-	-	-	-	-
LB18	-	-	-	-	-	-	-	-
LB19	-	-	-	-	-	-	-	-
LB20	-	-	-	-	-	-	-	-
LB21	+	-	-	+	+	-	-	-
LB22	+	-	-	+	-	-	-	-
LB23	+	+	-	-	-	-	-	-
LB24	+	+	-	-	-	-	-	-
LB25	-	-	-	-	-	-	-	-

Table 6. Summary of biochemical and physiological properties of isolates.

Isolate code	Heat survival at 60ºC			Heat survival at 65ºC			Gas from citrate	Ammonia from arginine
	30min	60min	90min	30min	60min	90min	-	-
LB26	-	-	-	-	-	-	-	-
LB27	-	-	-	-	-	-	-	-
LB28	-	-	-	-	-	-	-	-
LB29	+	-	-	-	-	-	-	-
LB30	-	-	-	-	-	-	-	-
LB31	+	+	-	-	-	-	-	-
LB32	-	-	-	-	-	-	-	-
LB33	-	-	-	+	+	-	-	-
LB34	-	-	-	-	-	-	-	-
LB35	-	-	-	+	+	-	-	-
LB36	+	-	-	-	-	-	-	-
LB37	-	-	-	-	-	-	-	-
LB38	-	-	-	-	-	-	-	-
LB39	+	-	-	-	-	-	-	-
LB40	-	-	-	-	-	-	-	-
LB41	-	-	-	+	-	-	-	-
LB42	-	-	-	-	-	-	-	-
LB43	-	-	-	-	-	-	-	-
LB44	+	-	-	-	-	-	-	-
LB45	-	-	-	-	-	-	-	-
LB46	+	+	-	-	-	-	-	-
LB47	-	-	-	+	-	-	-	-

Table 7. Summary of biochemical and physiological properties of isolates

Isolate code	Heat survival at 60ºC			Heat survival at 65ºC			Gas from citrate	Ammonia from arginine
	30min	60min	90min	30min	60min	90min	-	-
ST1	-	-	-	-	-	-	-	-
ST2	-	-	-	-	-	-	-	-
ST3	-	-	-	-	-	-	-	-
ST4	+	+	-	-	-	-	-	-
ST5	-	-	-	-	-	-	-	-
ST6	+	+	-	+	+	-	-	-
ST7	+	+	-	-	-	-	-	-
ST8	-	-	-	+	+	-	-	-
ST9	-	-	-	+	+	-	-	-
ST10	-	-	-	+	+	-	-	-
ST11	+	+	-	-	-	-	-	-
ST12	-	-	-	-	-	-	-	-
ST13	-	-	-	-	-	-	-	-
ST14	+	+	-	-	-	-	-	-
ST15	-	-	-	-	-	-	-	-
ST16	-	-	-	+	+	-	+	+
ST17	-	-	-	-	-	-	-	-
ST18	-	-	-	-	-	-	-	-
ST19	+	-	-	-	-	-	+	-
ST20	-	-	-	-	-	-	+	+
ST21	+	+	-	-	-	-	-	-
ST22	-	-	-	+	+	-	+	+
ST23	+	+	-	-	-	-	-	-

Table 8. Sugar fermentation

Isolate code	Sugar utilization					
	Lactose	Glucose	Galactose	Fructose	Mannose	Sucrose
LB1	+	-	-	+	+	-
LB2	+	-	+	+	+	-
LB3	+	-	+	+	+	-
LB4	+	-	-	+	+	+
LB5	+	+	-	+	+	+
LB6	+	+	-	+	+	-
LB7	+	-	+	+	+	-
LB8	+	-	+	+	+	-
LB9	+	+	+	+	+	-
LB10	+	+	+	+	+	-
LB11	+	+	-	+	+	+
LB12	+	-	-	+	+	+
LB13	+	-	-	+	+	+
LB14	+	-	-	+	+	+
LB15	+	+	-	+	+	+
LB16	+	-	-	+	+	+
LB17	+	+	+	+	+	+
LB18	+	+	+	+	+	-
LB19	+	+	+	+	+	-
LB20	+	+	+	+	+	-
LB21	+	-	-	+	+	+
LB22	+	-	-	+	+	-
LB23	+	+	-	+	+	+
LB24	+	+	-	+	+	-
LB25	+	-	-	+	+	-

Table 9. Sugar fermentation

Isolate code	Sugar utilization					
	Lactose	Glucose	Galactose	Fructose	Mannose	Sucrose
LB26	+	-	-	+	+	-
LB27	+	-	+	+	+	-
LB28	+	-	+	+	+	-
LB29	+	-	+	+	+	-
LB30	+	-	-	+	+	-
LB31	+	-	-	+	+	-
LB32	+	-	-	+	+	-
LB33	+	-	-	+	+	-
LB34	+	+	-	+	+	-
LB35	+	-	+	+	+	-
LB36	+	+	-	+	+	+
LB37	+	-	+	+	+	-
LB38	+	-	-	+	+	-
LB39	+	-	-	+	+	-
LB40	+	+	+	+	+	-
LB41	+	-	+	+	+	-
LB42	+	-	+	+	+	+
LB43	+	-	+	+	+	-
LB44	+	-	+	+	+	-
LB45	+	-	+	+	+	-
LB46	+	-	-	+	+	+
LB47	+	-	-	+	+	-

ability of growing at different temperature, for the bacilli identification the growth at 15 and 45°C and for classification of cocci isolates the growth ability at 10 and 45°C were assessed .*b.delbrueckii ssp.bulgaricus*cannot grow at 15°C and *s.thermophilus* can nt grow well at 45°C[3, 14].

According to the expect , all of the isolates grow well at 45°C and non of the bacilli and cocci isolates were able to grow at 15°C and 10°C respectively. 42 and 37°C are the best temperature for the best growing in lactobacillus and 42°C is the best for cocci ($p \leq 0.05$)(Table 2,3,4).

According to heat survival tests a large number of strains survived at 60 and 65°C for 30 min, only a few of them survived in 60 and 65°Cfor 60min, while all of them were destroyed in 60 and 65°C for 90 min($p \leq 0.05$) (Table 5,6,7).

Inability to produce gas from citrate was characteristic of the most of the strain and only 4 strains that produce gas from glucose before are able to produce gas from citrate too, and showed Heterofermentative behavior stronger than before (Table 5,6,7).

The most of the strain is powerlessness to produce ammonia from arginine and solitary,some of cocci consist of ST16,ST20 and ST22 were able to produce ammonia.

These isolates had been able to produce gas from glucose before. The significance of correlation between the production of gas from glucose and ammonia production from arginine is not well known but it may be that both of glucose and arginine is a vital factor for a special process and lead to produce bold metabolism for organisms(Table5,6,7)[16, 19].

The most useful test for the determination of strain differences is carbohydrate fermentation. According to the results all of MRS isolates gave positive results with sugars glucose , fructose , lactose and mannose. However the results of M17 isolated strains were much more confusing and according to literature information on sugar fermentation. It seems that larg number of scientists are agree about positive fermentation of glucose, lactoseand fructoseand,mannose,galactose, sucrose fermentation found to be variable[15].

Due to the evaluation of data lactose, glucose and fructose positive strains were determined as *S.thermophilus* (Table 8, 9,10).

Table 10. Sugar fermentation

Isolate code	Sugar utilization					
	Lactose	Glucose	Galactose	Fructose	Mannose	Sucrose
ST1	+	+	-	+	+	-
ST2	+	+	+	+	-	-
ST3	+	+	-	+	-	-
ST4	+	+	-	+	-	-
ST5	+	+	-	+	-	-
ST6	+	+	-	+	-	-
ST7	+	+	-	+	-	-
ST8	+	+	-	+	-	-
ST9	+	+	-	+	-	-
ST10	+	+	+	+	-	-
ST11	+	+	-	+	-	-
ST12	+	+	+	+	-	-
ST13	+	+	-	+	+	-
ST14	+	+	-	+	+	-
ST15	+	+	-	+	-	-
ST16	+	+	-	+	-	-
ST17	+	+	-	+	-	-
ST18	+	+	-	+	-	-
ST19	+	+	-	+	-	-
ST20	+	+	+	+	-	-
ST21	+	+	-	+	-	-
ST22	+	+	-	+	-	-
ST23	+	+	-	+	-	-

CONCLUSION

Isolation and identification of fermented dairy products starter bacteria is actually the first step for the use of these starter miroorganisms in the dairy industry,therefore , they should also be screened for their technological and physiological properties.

Isolation , identification and industrial production and application of local starter bacteria as starter culture is very important not only for bringing local and traditional taste to industrial products but also for saving local bacteria that couse special flavor , aroma and structure in dairy products.

REFERENCES

1. Azadnia P, Jashni MK, Ghasemi MSA, Babaki AK, Zamani M, Taarof N. Isolation and Identification of Mesophilic Lactobacilli from Traditional Yoghurts of Tribes of Kazerun. *Journal of Animal and Veterinary Advances.* 2011; **10**(4):528-31.
2. Kimoto-Nira H, Aoki R, Mizumachi K, Sasaki K, Naito H, Sawada T, et al. Interaction between Lactococcus lactis and Lactococcus raffinolactis during growth in milk: Development of a new starter culture. *Journal of dairy science.* 2012; **95**(4):2176-85.
3. Tamime AY, Robinson RK. Yoghurt: science and technology: Woodhead Publishing; 1999.
4. Mathara JM, Schillinger U, Kutima PM, Mbugua SK, Holzapfel WH. Isolation, identification and characterisation of the dominant microorganisms of kule naoto: the Maasai traditional fermented milk in Kenya. *International Journal of Food Microbiology.* 2004; **94**(3):269-78.
5. Pourahmad R, Assadi MM. Use of isolated native yogurt starter cultures in yogurt production Shortend title: Yogurt production by native starter cultures.
6. Pourahmad R, Mazaheri Assadi M. Yoghurt production by Iranian native starter cultures. *Nutrition & Food Science*. 2005; **35**(6):410-5.
7. Pourahmad R, ASSADI M. Use of isolated autochthonous starter cultures in yogurt production. *International Journal of Dairy Technology.* 2007; **60**(4):259-62.
8. Vinderola C, Mocchiutti P, Reinheimer J. Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. *Journal of Dairy Science*. 2002; **85**(4):721-9.
9. Badis A, Guetarni D, Boudjema BM, Henni D, Kihal M. Identification and technological properties of lactic acid bacteria isolated from raw goat milk of four Algerian races. *Food Microbiology.* 2004; **21**(5):579-88.
10. Guessas B, Kihal M. Characterization of lactic acid bacteria isolated from Algerian arid zone raw goats\'milk. *African Journal of Biotechnology.* 2004; **3**(6):339-42.
11. Poullet B, Huertas M, Sánchez A, Cáceres P, Larriba G. Main lactic acid bacteria isolated during ripening of Casar de Cáceres cheese. *Journal of Dairy Research.* 1993; **60**(01):123-7.
12. Chou L-S, Weimer B. Isolation and characterization of acid-and bile-tolerant isolates from strains of Lactobacillus acidophilus. *Journal of Dairy Science*. 1999; **82**(1):23-31.
13. Beukes EM, Bester BH, Mostert JF. The microbiology of South African traditional fermented milks. *International Journal of Food Microbiology.* 2001; **63**(3):189-97.
14. Teuber M. Lactic acid bacteria. Biotechnology Set, *Second Edition.* 1993:325-66.
15. Erkuº O. Isolation, Phenotypic and genotypic characterization of yoghurt starter bacteria: Izmir Institute of technology; 2007.
16. Briges M. The classification of lactobacilli by means of physiological tests. *Journal of general microbiology.* 1953; **9**(2):234-48.
17. Gibson T, Abdel-Malek Y. The formation of carbon dioxide by lactic acid bacteria and Bacillus licheniformis and a cultural method of detecting the process. *J. Dairy Res.* 1945; **14**:35-44.
18. Robinson RK. Dairy microbiology handbook: the microbiology of milk and milk products: John Wiley & Sons; 2005.
19. Boone DR, Castenholz RW, Garrity GM, Brenner DJ, Krieg NR, Staley JT. Bergey's Manual® of Systematic Bacteriology: Springer Science & Business Media; 2005.