

## Isolation and Identification of Mesophilic Lactobacilli from Traditional Yoghurts of Tribes of Kazerun

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**Abstract:** Morphological, cultural, physiological and biochemical characteristics were employed to identify mesophilic lactobacilli isolated from yoghurt in different areas in Kazerun city, Fars province, Iran. From 15 yoghurt samples, a total of 220 mesophilic lactobacilli were determined. Additionally, the biochemical tests and API kit showed *Lactobacillus plantarum* 85 (38.63%), *Lactobacillus brevis* 76 (34.55%) and *Lactobacillus casei* subsp. *casei* 59 (26.82%). So, *Lactobacillus plantarum* were found to be the more dominant species. The current study constitutes the first step in the designing process of LAB starter cultures in order to protect the typical organoleptic characteristics of drinking yoghurt. However in the future, we can consider genetical characterization and selection of the most desirable strains which can assess their potential as starter cultures for commercial use.

**Key words:** Lactic acid bacteria, mesophilic lactobacillus, yoghurt, dominant species, commercial use, Iran

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### INTRODUCTION

Interest in microorganisms as a component of biological diversity has been renewed in recent years (Guessas and Kihal, 2004). The interest in microorganisms occurring in foods is primarily due to the biotechnological potential of new bacterial species and strains (Leisner *et al.*, 1999).

Lactic Acid Bacteria (LAB) are widely distributed in nature and occur naturally as indigenous microflora in raw milk, yoghurt, etc. They are gram positive bacteria that play an important role in many food fermentation processes. Some species of the genus *Lactobacillus* (Lb.) are included in this group. The lactic acid fermentation has long been known and applied by humans for making different food stuffs. For many centuries, LAB have been an effective form of natural preservation. In addition, they strongly determine the flavor, texture and frequently, the nutritional value of food and feed products. However, the application of well-studied starter cultures has been established for decades (Lee, 1996; Tserovska *et al.*, 2002).

In the country, there are different kinds of traditional dairy products which are produced from sheep and goat milk such as drinking yoghurt, yoghurt, kashk, ghara-ghooroot, cheese, etc. In comparison with the

commercial species, composition of lactic acid bacteria is more varied and inconstant in these products. The aim of the present study was isolation and identification of mesophilic lactobacilli from yoghurt in order to constitute an original collection of Kazerun mesophilic lactobacilli strains.

### MATERIALS AND METHODS

**Yoghurt samples:** During the spring of 2009, a total of 15 yoghurt samples were collected from the tribes of Kazerun city, Fars province. The samples were collected in sterile universal tubes and kept cool until they could be taken to the laboratory where they were kept at 4°C for further use.

**Isolation of mesophilic lactobacilli:** The samples were aseptically weighted and homogenized. From each sample, a 1:10 dilution was subsequently made using peptone water followed by making a 10 fold serial dilution. 0.1 mL from each dilution was then subcultured in duplicate into the MRS agar (Merck, Germany) used for isolating lactobacilli (Badis *et al.*, 2004a; Guessas and Kihal, 2004). To prevent the growing of yeasts, the media were then supplemented with 100 mg L<sup>-1</sup> of cycloheximide before being incubated at the appropriate

temperatures (35°C) for 2-3 days (Beukes *et al.*, 2001; Kalavrouzioti *et al.*, 2005). MRS agar plates were incubated anaerobically using the Gas Pack system (Merck Anaerocult type A) at 35°C for 2-3 days in order to provide an optimal temperature for growing mesophilic lactobacilli.

Colonies were randomly selected and streak plating was then used to purify the strains which were subsequently kept in two different conditions including at 4°C for MRS plates and at -20°C for MRS broths supplemented by 20% glycerol for further use (Mathara *et al.*, 2004).

**Identification of the bacterial strains:** All strains were initially tested for gram reaction, catalase production and spore formation (Harrigan and McCance, 1976). Colonies were characterized on MRS agar. Strains with gram positive and catalase negative reactions were finally used for further identification (Sharpe, 1979). Growth at different temperatures (10, 15, 37, 40 and 45°C) for 5 days, resistance to 60°C for 30 min (Sherman test), growth in the presence of 2, 3, 4 and 6.5% NaCl and different pHs (4.5 and 6.5) were considered to identify the strains. Hydrolysis of arginine and aesculin, utilization of citrate, production of acetone, gas formation from glucose and dextran production from sucrose were also determined (Samelis *et al.*, 1994). All strains were also tested for fermentation of L-arabinose, D-xylose, galactose, D-fructose, sorbitol, lactose, melibiose, saccharose, D-raffinose, melezitose, mannose and glucose (Tserovska *et al.*, 2002).

The growth of bacterial strains at 10, 15, 37, 40 and 45°C was visually confirmed by the changes in turbidity of MRS broth after 24, 48 and 72 h of incubation. The tolerance of microorganisms to the different levels of salt, pH and heat (60°C) was also visually evaluated (Harrigan and McCance, 1976). Arginine dihydrolase agar and aesculin azid agar (Merck, Germany) were employed to perform the hydrolysis tests. For evaluation of citrate utilization and acetone production, citrate and MR-VP agars (Merck, Germany) were used. MRS broths containing inverted Durham tubes were used for evaluation of gas production and the production of dextran from sucrose was done in MRS agar (Mayeux *et al.*, 1962).

In order to assess the fermentation of sugars, a medium with the following composition was employed (g L<sup>-1</sup>): bovine extract, 10.0; neopepton, 10.0; yeast extract, 5.0; K<sub>2</sub>HPO<sub>4</sub>, 2.0; CH<sub>3</sub>COONa + 3H<sub>2</sub>O, 5.0; diammonium citrate, 2.0; MgSO<sub>4</sub>, 0.2; MnSO<sub>4</sub>, 0.05; brom-cresol-purple, 0.17; tween 80, 1 mL. Carbon sources were added individually to this medium as filter-sterilized

solutions to a final concentration of 1%. Carbohydrate utilization was assessed at the 24 and 48th h and on the 7th day of the growth at the corresponding temperature (Tserovska *et al.*, 2002).

Furthermore, sugar fermentation patterns of 80 strains were also tested by use of API (bioMérieux, France) 50 CH strips and API CHL medium. The tests were done according to the instructions of the manufacturer. Anaerobiosis in the inoculated strips was obtained by overlaying with sterile paraffin oil and incubated at 30-36°C and the results were read after incubation of the strains for 1-3 days. Identification was done by the computerized database program (version 5.1) provided by the manufacturer.

## RESULTS AND DISCUSSION

All 220 g positive, catalase negative and non spore-forming isolates were further divided into two subgroups according to Stiles and Holzappel (1997) as follows (Table 1):

- Mesophilic facultative hetero-fermentative Lactobacilli (210 isolates) which included *Lb. plantarum* (85 isolates, 38.63%) and *Lb. casei* subsp. *casei* (59 isolates, 26.82%)
- Mesophilic obligate hetero-fermentative Lactobacilli (76 isolates). *Lb. brevis* (76 isolates, 34.55%) was included in this group

It was noted that the mesophilic facultative hetero-fermentative lactobacilli group was represented by two species; 85 isolates were identified as *Lb. plantarum* and 59 isolates as *Lb. casei* subsp. *casei* according to Collins *et al.* (1989).

The last species is included in starter cultures during the production of the cheese Gruyere, Gorgonzola and Mozzarella (Tserovska *et al.*, 2002). The results are in accordance with other research groups in raw goat milk (Guessas and Kihal, 2004).

*Lb. plantarum* was also the major lactobacillus species found in kule naoto, the maasai traditional fermented milk (Mathara *et al.*, 2004). Moreover in the second group, a supplementation test for mannose and melizitose fermentation, permitted the identification of 76 isolates as *Lb. brevis* (Samelis *et al.*, 1994). Olarte *et al.* (2000) noted that the presence of *Lb. plantarum* in the cheese (Cameros) from goat milk decreased the number of the enterobacteriaceae and fecal coliforms in the final product. Lactobacilli isolated from household bushera belonged to *Lb. plantarum* and *Lb. brevis* (Muyanja *et al.*, 2003). In the characterization of microflora

Table 1: Physiological and biochemical characteristics of isolated strains

Characteristics	Strains		
	1	2	3
Gram stain reaction	+	+	+
Catalase activity	-	-	-
Glucose fermentation	+	+	+
NH <sub>3</sub> from arginine	-	+	-
<b>Growth at temperature (°C)</b>			
10	+	+	-
15	+	+	+
37	+	-	-
45	-	-	-
<b>Growth in a medium with NaCl (%)</b>			
2	+	+	+
3	+	-	+
4	+	-	+
6.5	-	-	-
<b>Growth at pH</b>			
4.5	+	+	+
6.5	+	+	+
Production of CO <sub>2</sub> from glucose	-	+	-
Dextran production	-	-	-
Acetoin production	+	-	-
Citrate hydrolysis	+	-	-
Heat resistance 63.5°C for 30	-	-	-
<b>Acid production</b>			
Arabinose	+	+	-
Esculin	+	-	+
Fructose	+	+	+
Galactose	+	+	+
Glucose	+	+	+
Lactose	+	+	+
Mannose	+	+	+
Melezitose	+	-	+
Melibiose	+	+	+
Raffinose	+	+	+
Sorbitol	+	+	+
Sucrose	+	+	+
Xylose	+	+	+

1 = *Lactobacillus plantarum*, 2 = *Lactobacillus brevis*, 3 = *Lactobacillus casei* subsp. *casei*

of homemade semihard white zlatar cheese, *Lactobacillus brevis* was found as one of the main groups (Terzic-Vidojevic *et al.*, 2007).

As starter cultures, lactobacilli are omnipresent in dairy manufacturing and specific fermentation processes have been developed in order to encourage the growth of the desired species (Bottazzi, 1988).

Isolates belonging to the *Lb. plantarum* group were shown to be the predominant members of the LAB flora of acid-fermented condiment (Tempoyak). In addition, isolates belonging to the *Lb. brevis* group were also observed (Leisner *et al.*, 2001). Beukes *et al.* (2001) found *Lb. plantarum* as dominant microorganisms of South African traditional fermented milks.

The most abundant isolated species from raw goat's milk of four Algerian races were *Lb. plantarum*, *Lb. brevis* and *Lc. lactis* subsp. *lactis* (Badis *et al.*, 2004b). Leisner *et al.* (1999) identified Lactic Acid Bacteria (LAB) of Chili Bo and found *Lb. plantarum* to be the most important predominant organism.

## CONCLUSION

Isolation and identification of Kazerun traditional yoghurt has been conducted for the first time. There is no record in the literature to demonstrate the isolation and identification of the Kazerun traditional yoghurt, so far. There is however, a big economic loss due to the import of yoghurt starters, annually. Because of increased demands for traditional fermented products, the results of the present study might be able to launch a considerable native achievement in the production of yoghurt. The identified isolates are used to establish the production of volatile compounds and to assess their potential as starter cultures for their commercial uses.

## ACKNOWLEDGEMENTS

We acknowledge the Islamic Azad University, Kazerun Branch and Shiraz Veterinary Administration who technically supported this research.

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