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

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**Abstract:** Morphological, cultural, physiological and biochemical characteristics were employed to identify thermophilic lactobacilli isolated from yoghurt in different areas in Kazerun city, Fars province, Iran. From 15 yoghurt samples a total of 99 thermophilic Lactobacilli were determined. Additionally, the biochemical tests and API kit showed *Lactobacillus helveticus* 25 (25.25%), *Lactobacillus fermentum* 20 (20.20%) and *Lactobacillus delbrueckii* sp. *bulgaricus* 54 (54.55%). So, *Lactobacillus delbrueckii* subsp. *bulgaricus* were found to be the more dominant species. The current study constitutes the first step in the designing process of thermophilic lactobacilli starter cultures, in order to protect the typical organoleptic characteristics of yoghurt. However, in the future can consider genetical characterization and selection of the most desirable strains which can assess their potential as starter cultures for commercial use.

**Key words:** Isolation, identification, thermophilic lactobacilli, traditional yoghurt, Kazerun, Iran

## 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 thermophilic lactobacilli from yoghurt in order to constitute an original collection of Kazerun thermophilic 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 thermophilic lactobacilli:** The samples were aseptically weighted and homogenazied. From each sample, a 1:10 dilution was subsequently made using peptone water followed by making a 10 fold serial dilution. About 0.1 mL from each dilution was then subcultured, in duplicate into the MRS agar (Merck, Germany) used for isolating Lactobcilli (Badis *et al*., 2004a; Guessas and Kihal, 2004). To prevent the growing of yeasts, the media were then supplemented with 100 mg $L^{-1}$ of cycloheximide before being incubated at the appropriate temperatures (42°C) for 2-3 days (Beukes *et al*., 2001; Kalavrouzioti *et al*., 2005). MRS agar plates were incubated anaerobically using the gas pack system

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(Merck Anaerocult type A) at 42°C for 2-3 days in order to provide an optimal temperature for growing thermophilic lactobacilli. Colonies were randomly selected and streak plateing was then used to purify the strains which were subsequently kept in 2 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 MaCance, 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 asculin, 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 MaCance, 1976). Arginine dihydrolase agar and asculin 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 $L^{-1}$): bovine extract, 10.0; neopepton, 10.0; yeast extract, 5.0; $K_2HPO_4$, 2.0; $CH_3COONa+3H_2O$, 5.0; diamonium citrate, 2.0; $MgSO_4$, 0.2; $MnSO_4$, 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 48 h and on the 7th day of the growth at the corresponding temperature (Tserovska *et al*., 2002). Furthermore, sugar fermentation patterns of 50 strains were also tested by use of API (bioMerieux, 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 36°C and the results were read after incubation of the strains for 1, 2 and 3 days. Identification was done by the computerized database program (version 5.1) provided by the manufacturer.

## RESULTS

All 99 g positive, catalase negative and non spore-forming isolates were further characterized as Thermophilic obligate homo-fermentative lactobacilli according to Stiles and Holzapfel (1997), including *Lb. helveticus* (25 isolates, 25.25%), *Lb. fermentum* (20 isolates, 20.20%) and *Lb. delbrueckii* sp. *bulgaricus* (54 isolates, 54.55%) which had a narrow fermentation profile and was able to ferment lactose and fructose and thus would likely belong to *Lactobacillus delbrueckii* sp. *bulgaricus* (Samelis *et al*., 1994; Guessas and Kihal, 2004) (Table 1).

Table 1: Physiological and biochemical characteristics of isolated strains

| Characteristics | 1 | 2 | 3 |
|---|---|---|---|
| Gram stain reaction | + | + | + |
| Catalase activity | - | - | - |
| Glucose fermentation | + | - | + |
| $NH_3$ from arginine | - | - | - |
| **Growth at temperature (°C)** | | | |
| 10 | - | - | - |
| 15 | - | - | - |
| 37 | + | + | + |
| 45 | + | + | + |
| **Growth in a medium with NaCl (%)** | | | |
| 2 | + | + | + |
| 3 | + | + | + |
| 4 | + | + | + |
| **Growth at pH** | | | |
| 6.5 | + | + | + |
| 4.5 | + | + | + |
| 6.5 | - | - | - |
| Production of $CO_2$ from glucose | - | - | - |
| Dextran production | - | - | - |
| Acetoin production | - | - | - |
| Citrate hydrolysis | - | - | - |
| Heat resistance 63.5°C for 30' | + | - | + |
| **Acid production** | | | |
| Arabinose | - | - | - |
| Esculin | - | - | + |
| Fructose | - | + | + |
| Galactose | + | - | + |
| Glucose | + | - | + |
| Lactose | + | + | + |
| Mannose | - | | - |
| Melezitose | - | - | - |
| Melibiose | - | - | - |
| Raffinose | - | - | - |
| Sorbitol | - | - | - |
| Sucrose | - | - | + |
| Xylose | - | - | - |

1 = *Lactobacillus helveticus*, 2 = *Lactobacillus delbrueckii* sp. *bulgaricus*, 3 = *Lactobacillus fermentum*

## DISCUSSION

It was noted that the thermophilic facultative hetero-fermentative lactobacilli group was represented by 3 species; 25 isolates were identified as *Lb. helveticus*, 20 as *Lb. fermentum* and 54 isolates as *Lb. delbrueckii* ssp. *bulgaricus* according to Samelis *et al*. (1994).

*Lb. helveticus* is included in starter cultures during the production of the cheese Gruyere, Gorgonzola and Mozarella (Tserovska *et al*., 2002). The above-mentioned results are in accordance with other research groups, in raw goat milk (Guessas and Kihal, 2004). Lactobacilli isolated from household bushera belonged to *Lb. plantarum, Lb. brevis and Lb. delbrueckii* sp. *bulgaricus* (Muyanja *et al*., 2003). As starter cultures, LAB are omnipresent in dairy manufacturing. Specific fermentation processes have been developed in order to encourage the growth of the desired species, some of which are fastidious organisms such as *Lb. delbrueckii* sp. *bulgaricus* and *Lb. helveticus* (Bottazzi, 1988).

Beukes *et al*. (2001) found *Lb. delbrueckii* sp. *bulgaricus* as dominant microorganism of South African traditional fermented milks. The most abundant isolated species from raw goat's milk of four Algerian races were *Lb. helveticus* and *Lb. delbrueckii* sp. *bulgaricus* (Badis *et al*., 2004b).

## 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.

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