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Isolation and Identification of Lactococci from Traditional Yoghurt in Tribes of Kazerun

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Abstract: Morphological, cultural, physiological and biochemical characteristics were employed to identify Lactococci isolated from yoghurt in different areas in Kazerun city of Fars province in Iran. From 15 yoghurt samples a total of 28 Lactococci were determined. Additionally, the biochemical tests and API kit showed that all of them were *Lactococcus lactis* subsp. *cremoris*. The current study constitutes the first step in the designing process of LAB starter cultures, in order to protect the typical organoleptic characteristics of traditional 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, lactococci, 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 *Lactococci* (Lc.) 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 Lactococci from yoghurt in order to constitute an original collection of Fars province Lactococci strains.

MATERIALS AND METHODS

Yoghurt samples: During the spring of 2009, a total of 15 yoghurt samples were collected from the tribes of Kazerun. 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 lactococci: 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. About 0.1 mL from each dilution was then subcultured, in duplicate into the M17 agar (Merck, Germany). 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 (30°C) for 2 days (Beukes *et al*., 2001; Kalavrouzioti *et al*., 2005). M17 agar plates were incubated aerobically at 30°C for 2 days, in order to provide an optimal temperature for growing Lactococci.

To perform the counts, the higher dilutions were used. Colonies were randomly selected and streak plating was then used to purify the strains which were subsequently kept in 2 different conditions including at 4°C for M17 agar plates and at -20°C for M17 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

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spore formation (Harrigan and MaCance, 1976). Colonies were characterized on Acetate 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 or M17 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 1-3 days. Identification was done by the computerized database program (version 5.1) provided by the manufacturer.

RESULTS

All 28 gram positive, catalase negative and non spore-forming isolates were further characterized as mesophilic homo-ferme ntative cocci. This group was represented by $ADH^{(-)}$ (arginine dihydrolase) (negative arginine hydrolysis), $citrate^{(-)}$ (negative citrate utilization) and $acetoin^{(-)}$ (negative acetoin production) isolates which were identified as *Lactococcus lactis* subsp. *cremoris* (Table 1). In this group, the microorganisms were spherical or ovoid in shape, occurring in pairs and short chains with non motile, facultative anaerobic fermentative metabolism (Holt, 1994).

Table 1: Physiological and biochemical characteristics of isolated strains

Characteristics of the srains	Results
Gram strain 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	V
4.5	-
6.5	+
Production of CO_2 from glucose	-
Dextran production	V
Acetoin production	-
Citrate hydrolysis	-
Heat resistance 63.5°C for 30'	
Arabinose	-
Esculin	-
Fructose	-
Galactose	-
Glucose	+
Lactose	+
Mannose	-
Melezitose	-
Melibiose	V
Raffinose	-
Sorbitol	-
Sucrose	-
Xylose	-

V = Variable

DISCUSSION

It was noted that the 28 isolates were identified as *Lactococcus lactis* subsp. *cremoris*. Beukes *et al*. (2001) found *Lc. lactis* as one of the dominant microorganisms of South African traditional fermented milks. One of the most abundant isolated species from raw goat's milk of four Algerian races were also *Lc. lactis* subsp. *lactis* (Badis *et al*., 2004). The low number of these lactic acid cocci is probably due to their inability to compete with lactic acid bacilli in mixed cultures (Teuber and Geis, 1981; Togo *et al*., 2002).

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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