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Antibacterial activity and cholesterol assimilation of lactic acid bacteria isolated from traditional Iranian dairy products

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ABSTRACT

In this study, lactic acid bacteria were isolated from ewe milk, traditional yoghurt and sour buttermilk samples collected from different areas of Azarbayjan-e-sharqi in Iran. All the isolates were screened for their ability to produce bacteriocin like inhibitory substances (BLIS) by studying their inhibitory action against pathogens like *Listeria monocytogenes*, *Salmonella enteritidis* and *Staphylococcus aureus*, after eliminating the effect of organic acids and hydrogen peroxide. According to results, four of the isolates identified as *Lactobacillus brevis, Lactobacillus pentosus, Pedoicoccus acidilactici* and *Lactobacillus paracasei* were unaffected by the action of pH neutralization and hydrogen peroxide and showed inhibitory action against the tested pathogens. The inhibitory activities demonstrated by these isolates were completely inhibited in the presence of proteolytic enzymes.

The isolates in study were further characterized for their cholesterol reduction ability. Cholesterol assimilation by both viable and dead cells of these strains was determined in MRS broth containing 0.3 g/100 mL bile salt. According to results, highest level of cholesterol removal was recorded in *L. brevis*, while all the other isolates in study were also able to reduce cholesterol to lesser extent. To conclude, the Lactic Acid Bacteria isolated from these traditional products might be exploited for their probiotic potential for future studies.

1. Introduction

Elie Metchinkoff, the Russian physician who proposed the concept of probiotic, propounded that the longevity of the Bulgarians was in part due to consumption of large quantities of fermented milk containing *lactobacilli* (Metchnikoff, 1908). *Lactobacilli* and *Bifidobacter* are two kinds of Lactic Acid Bacteria (**LAB**), which are found in the gut and are considered as probiotic, because of their beneficial effects on health (Mitsuoka, 1998). Later, Fuller (1989) defined probiotics as live, non-pathogenic bacteria that contribute to the health and balance of the intestinal tract.

Fermented foods are known throughout the world, and the Gram positive LAB have been well known for their important role in food industry (Karimi Torshizi, Rahimi, Mojgani, Esmaelkhanian, & Grimes, 2008). These bacteria produced antimicrobial agents such as acids, hydrogen peroxide and bacteriocins and have great potential as food bio-preservatives (Aslim, Yukesekdag, Sarikaya, & Beyatli, 2005; Avonts, Uytven, & Vuyst, 2004). Bacteriocins are ribosomal synthesized proteinaceous substances produced by LAB, containing relatively narrow spectrum of bactericidal activity (Cleveland, Montville, Nes, & Chikindas, 2001).

In recent years, consumption of fermented foods has increased due to the reported beneficial health effects of LAB including lowering of serum cholesterol level. Various studies *in vivo* have shown that some *Lactobacilli* can lower cholesterol (Anderson & Gilliland, 1999). Numerous health benefits of LAB have made them promising probiotic candidates and being extensively studied to explore their safety and other desirable properties. The main objective of the present study was to investigate and characterize Lactic Acid Bacteria in traditional sour buttermilk made from ewe's milk, which might provide important information regarding its probiotic potential and its utilization in future.

2. Materials and methods

2.1. Bacterial strains and culture conditions

All LAB strains used in this study were grown in MRS broth (de Man, Rogosa Sharpe, HiMedia, Mumbai, India) at 37 °C for 24–48 h in anaerobic jars, pathogenic strains used in this study were grown

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in BHI (Brain–heart infusion broth, HiMedia, Mumbai, India) at 37 °C for 18–24 h under aerobic condition.

All LAB strains were maintained at 4 °C and renewed every week for short-term preservation. The long-term conservation of the purified isolates was carried out in MRS broth with sterile glycerol (15 mL/100 mL) and stored at −70 °C (Badis, Guetarni, Boudjema, Henni, & Kihal, 2004).

2.2. Collection of ewe milk and preparation of buttermilk

A total of 20 samples from ewe milk were analyzed, of which 10 samples were collected from Myaneh (15 herds) and 10 samples obtained from Hashrood (15 herds) two cities in Azarbayjan-e-sharqi (north-west of Iran). In both places, samples were taken at 10 different days and collected from bulk milk tank. All samples were collected according to EN ISO 707:2001 in sterile bottles of 250 mL and transported to the laboratory under refrigeration (4 °C) within 36 h (Anon, 2002).

The traditionally made yoghurt (n: 20) and sour buttermilk (n: 20) samples which made from ewe milk, were also collected from the same area. Fig.1 shows the preparation operation of yoghurt and sour buttermilk. As indicated, the samples are prepared in a traditional device known as Mashk, which is made from hide (sheepskin) and is used for making butter and buttermilk from yoghurt. This device is also used widely for preservation of fermented dairy products for 10–20 days at temperatures not exceeding 20 °C.

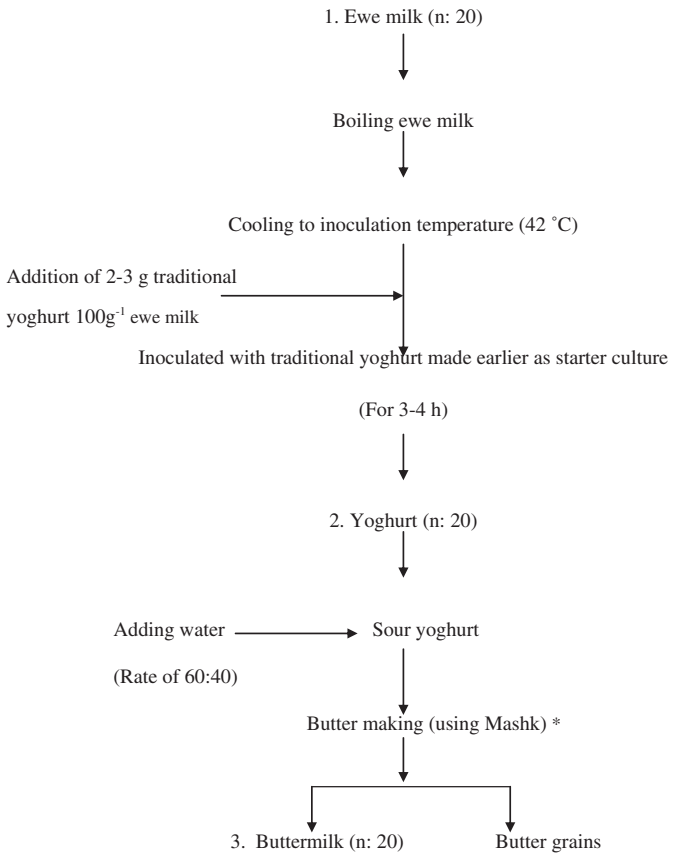

Fig. 1. Preparation of samples including ewe milk, yoghurt and traditional sour buttermilk. *(Mashk is a traditional device used in this study, which is made from hide and is used for making butter and buttermilk from yoghurt or keeping fermented dairy products).

2.3. Isolation and identification of LAB

One ml of each sample (ewe milk, yoghurt and sour buttermilk) was inoculated in MRS broth under above mentioned conditions (part 2.1) until growth was observed. The grown samples were then plated on MRS agar and incubated at 37 °C until appearance of colonies. Pure colonies were selected and tested for Gram staining, cell morphology and catalase test (Karimi Torshizi et al., 2008).

2.4. Identification of isolated LAB to species level

Only strains showing maximum inhibitory activity against others (4 selected lactic acid bacteria) were selected for identification to species level. The carbohydrate fermentation profiles of the selected strains were investigated using API 50 CHL medium (Bio-Meriux, Marcy l'Etoile, France) according to the manufacture's instruction (Iranmanesh et al., 2012).

2.5. Antimicrobial activity of supernatant against pathogens

The antimicrobial effects of selected LAB against Gram positive and Gram negative pathogens were examined by agar well diffusion methods. The pathogens namely *Staphylococcus aureus* (PTCC 1112), *Listeria monocytogenes* (PTCC 1298), and *Salmonella enteritidis* (local isolate) were used as indicator culture in the study. The culture broths of the producer and indicator strains were adjusted to McFarland Index 3 prior to use. The antimicrobial activity was recorded as appearance of clear zone around the wells (Aslim et al., 2005).

2.6. Bacteriocin production

Cell free supernatant of the selected LAB strains was obtained by centrifuging the culture broth at 10,000 g for 10 min at 4 °C, adjusted to pH 6.5 with 1 mol equi/L NaOH followed by filtration through a 0.22 μm pore size filter. To eliminate the effect of hydrogen peroxide, catalase was added at a final concentration of 1 mg/mL to the supernatant broth and the remaining activity determined as described earlier (Ghrairi, Manai, Berjeaud, & Frere, 2004).

2.7. Sensitivity of bacteriocin-like substance to enzyme

The supernatant of selected strains was treated with the following enzymes (Sigma–Aldrich, St. Louis, Mo, USA) at a final concentration of 1 mg/100 mL: lysozyme, pronase, trypsin and proteinase K. The treated samples were incubated at 37 °C for 2 h and the remaining activity determined against the listed indicator strains (Bromberg, Moreno, Lopez Zaganini, Regina Delboni, & De oliveria, 2004).

2.8. Cholesterol removal by viable and dead cell

Water-soluble cholesterol was filter-sterilized and added at a final concentration of 70 μL/100 mL to MRS broth containing 0.3 g/100 mL oxgall (Sigma–Aldrich, St. Louis, Mo, USA). The tube was inoculated with each selected strains (at 1 mL/100 mL) and incubated at 37 °C for 2, 4, 9 and 24 h. After incubation, the cells were centrifuged at 10,000 g for 10 min.

For preparation of heat-killed cells, the cell pellet of 5 selected strains with the highest effect of removing cholesterol was washed twice with 10 mL of sterile distilled water and autoclaved for 15 min at 121 °C, then diluted with ringer solution and counted by microscope. The heat-killed cells were suspended in MRS broth containing 0.3 g/100 mL oxgall and water-soluble cholesterol