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Phenotypic Characterization of Lactic Acid Bacteria Isolated from Traditional *Koopeh* Cheese

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Abstract: The aim of this study was to isolate and identify phenotypic characterization of isolated lactic acid bacteria from *Koopeh* cheese, a traditional jug cheese with unique manufacturing method, produced in West Azerbaijan province of IRAN. A total number of 24 samples of *Koopeh* cheese was collected randomly in ten cities of West Azerbaydjan province. After preparing of cheese samples, serial dilution and plating on MRS and M17 agar for lactobacillus and lactococci isolation, respectively were carried out. Isolated Gram positive and catalase negative colonies, based on their physicochemical properties were analyzed. A total of 501 isolates were examined. Average total bacterial count of cheese samples was 6.9×10^6 CFU/gm and the average population of Lactic acid bacteria was 4.6×10^5 CFU/gm. Most of lactic acid bacteria were cocci (72.4 %) and bacilli (27.6 %). Dominant coccus isolate were *Enterococcus* (52 % of lactococci population). Dominant isolated Bacillus genus was *Lactobacillus plantarum* (58% of lactobacilli population).The results of the present study showed that characteristics of *Koopeh* cheese are unique and this traditional dairy product has specific microbial populations. All isolates of *Koopeh* cheese were homofermentative or probably facultative heterofermentative. It's suggested that molecular methods are better for identification of isolated lactic acid bacteria of *Koopeh* cheese.

Key words: *Koopeh* cheese · Lactic acid bacteria · Lactobacillus · Lactococcus · Phenotyping

INTRODUCTION

Lactic acid bacteria as a widely spread great natural group of bacteria are the main microflora of raw milk, milk products and many fermented foods [1-2]. This group of bacteria have the main role in production and ripening of the various types of cheeses and are used as natural or selective starter in food fermentation [3-4]. Lactic acid bacteria as a group of Gram-positive, catalase-negative, non spore forming and anaerobic organisms with, low G + C are eligible for symbiotic life. These bacteria with different genera, have probiotics strains. These genera include 11 genus that six of them are seen in dairy products: Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus and Pediococcus. Lactic acid bacteria based on the metabolism of carbohydrates are divided into two categories homofermentative and hetrofermetative [1, 5, 6]. Fermentation is one of the old methods for food preservation, mainly by lactic acid bacteria (LAB) that cause production of lactic acid, acetic acid, ethanol, aromatic compounds, exopolysacharides and bacteriocins [7]. Fermented foods preparation are mainly traditional that help to increase product shelf life, especially in areas with no possibility of using modern methods of food storage [8]. Cheese as a milk fermentation product has high diversity. Lactic acid bacteria are main population in the microorganisms of cheese, that in most cheeses, are used as starter bacteria [9].

Cheeses made traditional from raw and unpasteurized milk have a rich and diverse microflora. Environment plays a major role in traditional cheese fermentation and is an important indicator that affect in their quality [10]. Lactic acid bacteria are responsible for different functions in

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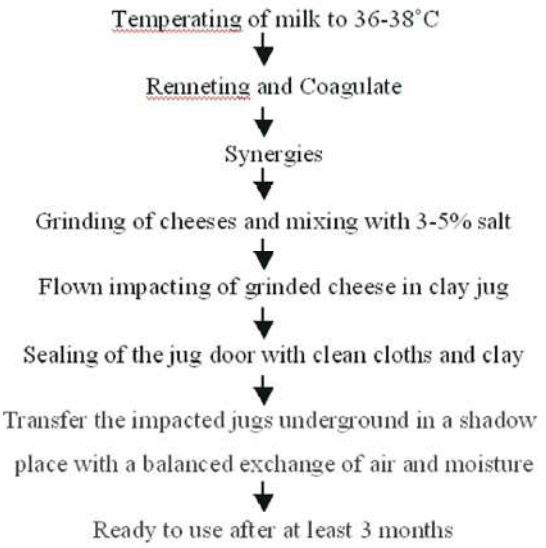

Fig 1: Koopeh cheese making method

cheese production. Some species cause conversion of milk lactose to lactic acid in the fermentation process, lactic acid bacteria with this ability named starter lactic acid bacteria (SLAB) which include Lactococcus lactis and Leuconostoc spp (Mesophiles) and Lactococcus delbrucki, Lactococcus helveticus and Streptococcus termophilus (Termophiles). Other group of LAB that contribute in ripening process of cheese are known as non-starter lactic acid bacteria (NSLAB) which include Lactobacillus spp. Pediococcus and Enterococcus [1]. Non-starter lactic acid bacteria are the main microflora of cheeses with long ripening period and are responsible for final ripening of cheeses [11].

Koopeh cheese as a semi-hard cheese produced traditionally from raw and unpasteurized sheep milk and rarely of caw milk in IRAN and its neighbor countries (TURKEY and IRAQ) [12]. *Koopeh* is a term that refers to jug in different areas of West Azarbaijan. Traditional methods of *Koopeh* cheese manufacturing is unique and different from brined cheese. Figure 1 shows the traditional production of *Koopeh* cheese step by step. At present there is no study about the lactic acid bacteria of *Koopeh* cheese in Iran. The aim of this study was enumeration, isolation and identification of different genera of lactic acid bacteria in traditional *Koopeh* cheese produced in different areas of West Azarbaijan province.

MATERIALS AND METHODS

Sample Collection: Twenty four samples of ripened *koopeh* cheese was purchased from ten cities of West Azerbaijan province of Iran (2 from each of Khoy, Salmas, Maku, Mahabad, Piranshahr, Bukan, Sardasht, Shahindejh, Oshnavieh and 6 samples from Orumieyeh). Cheese samples, weighing 250 grams were transported to laboratory in an ice box.

Methods: The *Koopeh* cheese making method was written based on surveys by the authors of the traditional cheese makers in different villages of the province. Ten Grams of each sample was weighed aseptically and transferred to sterile plastic bags and then homogenized in 90 ml of sterile sodium citrate 2% (w/v) at temperature of 45°C using Lab-Blender 400 Stomacher for 2 min with 200 rpm were homogenized, to obtain 1:10 dilution. Serial dilutions to 10^{-8}c were made in sterile 0.1% (w/v) peptone water (Merck) [10-13]. From each dilution 50 µl was plated in duplicates onto PCA[1], MRS[2] agar and M17 agar (Merck), for enumeration of total bacteria, lactobacilli and lactococci, respectively. PCA plates were incubated at 37°C for 72 h and MRS agar and M17 agar plates were incubated at 30, 35 and 42°C for 72 h in anaerobic conditions using Gas Pack System (Anaerocult C). Five colonies were picked from plates with 30-300 colonies, randomly [8]. Gram positive and catalase-negative colonies were purified by 2-3 times culturing on selective media. MRS agar medium containing vancomycin (20 µg/ml) was used for enumeration of Leuconostoc and incubated at 30°C and MRS agar medium containing vancomycin (20 µg/ml) + NaCl (5%) was used for enumeration of pediococcus. Bile esculin medium was used for enterococci detection [2, 9, 14-17].

Statistical Methods: The mean, standard deviation and the percentage of statistical data has been obtained using the statistical software Minitab (version 15). All experiments were repeated twice.

RESULTS AND DISCUSSION

A total of 720 bacterial isolates were examined of which, 501 isolates belonged to different genera of lactic acid bacteria. Total bacterial count of cheese samples was 9.6×10^6 CFU/gm and the average population of lactic acid bacteria was 6.4×10^5 CFU/gm (Table 1). Most of lactic acid

[1]Plate count agar
[2]Man rogosa sharpe agar

Table 1: Lactic acid enumeration at different temperature (CFU/gm)

Media No	Total count (PCA)	MRS 30°C a*	MRS 35°C an**	MRS 42°C an	MRS + VAN30°C a	M17 30°C a	M17 35°C an	M17 42°C a	Bile_ Esculin Agar an
Average	9.6×10^6	6.1×10^5	1.1×10^6	2.8×10^4	1.5×10^4	2.8×10^5	5.5×10^5	2.7×10^6	1.2×10^6
SD	1.6×10^6	3.4×10^5	1.1×10^6	2.5×10^4	-------	1.6×10^5	7.1×10^5	3.1×10^6	1.2×10^6

*a: aerobic *an: anaerobic

bacteria were cocci, 363 isolate (72.4 %) and 138 isolates were Bacilli (27.6 %). Dominant coccus isolates were *Enterococcus* (189 isolate), *Lactococcus lactis* (152 isolate), *Leuconostoc* (7 isolate) and *Pediococcus* (8 isolate). Dominant isolated lactobacilli were *Lactobacillus plantarum* (80 isolate), *Lactobacillus casei* (25 isolate), *Lactobacillus paracasei* and *L. Helveticus* (11 isolate). *Leuconostoc* strains were isolated only from 10 samples. Sixteen isolates of lactobacilli could not be detected.

In this research number of cocci significantly were greater than lactobacilli isolates ($P < 0.001$). Isolates were classified in three categories Mesophilic lactobacilli and cocci and thermophilus cocci. Many important factors affect cheese organoleptic characteristics such as: milk type, quality and microbial population, processing method and ripening condition of cheese. Lactic acid bacteria have the main role in production of aromatic compounds and flavor in cheese [18] producing regions of traditional cheeses and affect cheese microbial population diversity [19]. Unlike the industrial cheeses, starter cultures are not used in traditional cheese manufacturing, as a result producing methods of traditional cheese varied on the basis of cheese type. Exclusive processing methods of *Koopeh* cheese and long time maintenance of jugs contained cheese underground cause specific diversity of lactic acid bacteria. There are different studies about isolation and identification of lactic acid bacteria in traditional dairy products of IRAN which are produced from sheep, goat and caw milk such as yoghurt, kashk, drinking yoghurt, gharaghooroot and cheese [2-20] but there is no study about *Koopeh* cheese.

Lactobacillus plantarum significantly has comprised the majority of isolated lactobacilli ($P < 0.001$). Mesophilic cocci significantly was higher than thermophilic cocci ($P < 0.005$). In the present study, dominant lactobacilli in *Koopeh* cheese were *Lactobacillus plantarum* (58%) and *Lactobacillus casei* (18%) that is according to other reports of other cheese types [1-6-10-14-20-21].

Dominant isolated cocci were Enterococcus (52%) and *Lactococcus lactis* (42%). Enterococcus genus is naturally widely distributed in the environment; as a result its high number is most probable in dairy products. The main role of Enterococcus as a natural stater is because of their proteolytic and lipolytic abilities [17-22]. High number of enterococci was reported in raw milk sheep in Iran [23]. The results of this study is agree with other researches. Dominant population of Enterococci was found in Lighvan cheese [18-24], in São Jorge cheese from Portugal [25], in cebriero cheese, an Italian cheese [26], in Beyaz cheese from turkey [21] and in other cheeses such as Serra, Teleme, Feta, comte, Manchego, Mozzarella and Kefalotyric [27].

Isolated Pediococcus from *Koopeh* cheese had low abundance (2.2%). This genus of lactic acid bacteria is active on cheese ripening and fermentation test of lactose [28]. Their abundance in Lighvan cheese was higher (12%) [23].

In the current work, Leuconostocs had 2.8% abundance in lactic acid bacteria group of *Koopeh* cheese. They are the main group of LAB in most of various types of milks [6-29]. Østlie *et al.* [14] stated this group of LAB has complex nutrition requirements, that cause unsuccessful competition with other genera of LAB and low abundance in cheese. Leuconostocs are not proteolytic and can produce diacetyl, acetate and ethanol, which impress aroma and flavor of dairy products. All isolates of *Koopeh* cheese were homofermentative or probably facultative heterofermentative. Underground long ripening period of *Koopeh* cheese caused reduction in lactobacillus population (27%), comparing with other type of cheeses. Comparing of LAB genera in raw milk of different animals with LAB of ripened cheeses that are produced of them can help scientists to better understanding of what occur in ripening period.

The obtained results showed high diversity of LAB in *Koopeh* cheese that could cause popularity and favorable quality in this type of cheese.

In conclusion, its recommended that the population of LAB in raw sheep milk has to be studied and compared with *koopeh* cheese microflora and the role and function of each group of LAB and their impact's on cheese quality are investigated and discussed. Also, it is suggested that molecular methods are better for identification and classifying of lactic acid bacteria, because of less discriminative ability of classical methods.

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