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Original article

Isolation, identification and comparison of lactic acid bacteria from fermented be produced in Iran Kimchi with Korean commercial samples: introduction of a probiotic product

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ABSTRACT

Kimchi is the general term given to a group of acid fermented vegetable foods traditionally produced in Korea, China and other South Asian countries. In this study, two different formulations of Kimchi were produced in Iran. After acidification to pH=4.2, then LAB were isolated, enumerated and identified. Six strains of LAB were isolated from Kimchi during on the 3th day of fermentation processing with using MRS agar plates and then were on of identified on the basis of morphological, biochemical, and physiological characteristics. Isolates that were identified as *Leu.mesenteroides* (6.8%), *L.bervis* (11.28%), *L.fructosus* (13.5%), *L.plantarum* (16.5%), *L.homohiochii* (20.3%), and *L.fermentum* (31.6%) Thus, 93.2% of the isolates were *Lactobacillus* and the rest (6.8%) were *leuconostoc*. Also results showed that no significant difference between Iranian and Korean Kimchi about geniuses of LAB of Iranian and Korean Kimchi.

1. Introduction

Kimchi is a kind of traditional fermented foods in Korea, which is classified as pickled vegetables such assauerkraut. Chinese cabbage or radish is widely used as the main source of *kimchi* with various ingredients such as garlic, ginger and several fermented sea foods. Thetaste and fragrance of *kimchi* are chiefly dependent on the micro flora that change during the fermentation which result in the liberation of free sugars, amino acidsand organic acids. In particular, free sugars such as glucose, fructose and mannose liberated from cabbage or radish play important roles in the taste of kimchi because fermentation is mainly controlledby these sugars (Yun *et al.,* 1996). Kimchi, being a lactic acid-fermented vegetable product consumed raw, is therefore also considered to be a good source of potentially beneficial and useful LAB. Among these, particularly the *lactobacilli* are of interest as beneficial inhabitants of the intestinal tract of man and animals. A wide range of such desired roles have been suggested for diverse strains of LAB, including immune stimulation, pathogen exclusion, production of bioactive substances, and general intestinal health (Lee *et al.,* 2011 ; Heejae *et al.*, 2011). Nutritionally, kimchi is an important source of vitamins, minerals, dietary fiber, andother functional nutrients and phytochemicals. Kimchi might help to increase appetite, reduce constipation, control intestinal flora, and have anti carcinogenic and anti aging effects and other health benefits (park *et al.,* 2001). Traditionally in Korea, large quantities of kimchi are prepared as an annual event, kimjang, for eating during the winter when the fresh vegetable supply is limited. Most of the vegetables cultivated in Korea are used as sources for making kimchi. Although 161 or 187 kinds of kimchi are currently reported (Edward, 2008).

The purpose of this study was to determine the amount of micro flora of kimchi that were produced in Iran and Isolation and Identification Lactic Acid Bacteria (LAB) from it, then comparison then with koreian Kimchi Another purpose is studying Changes in microbiological composition of kimchi during fermentation the identity of the culture was based on characteristics of the Lactic Acid Bacteria as presented in Bergey's Manual of Determinative Bacteriology, also were studied carrying out microscopy (morphology), Gram straining, analysis growth at 15 and 45 °C, quality and quantitive fermentation processing in different carbon sources.

2. Materials and methods

2.1. Materials

In this study, the ingredients (Chinese cabbage, Leek, red pepper powder, Garlic, Ginger, Sugar, rice flour) were used in kimchi preparation and were purchased from local markets in Mashhad, Iran.

2.2. Production of kimchi

The preparation of kimchi was carried out based on the ‘‘baechu-kimchi’’ recipe. The ingredients are listed in Table 1. Previously, garlic, ginger, and leek were chopped. Sugar was weighed. The Chinese cabbage was cut into pieces 3 cm × 3 cm in length and soaked in 15% (w/v) brine for 30 min. The soaked cabbage was washed twice with fresh water and then drained for 30 min. The prepared ingredients were mixed well and then distributed evenly on the Chinese cabbage. The kimchi mixture was put into a jar. It was fermented at 22˚C for 3 days (Cho *et al.,* 1997; Cho *et al.,* 1998).

Table 1
Composition of kimchi.

Materials and ingredients	Percentage share
Salted Chinese cabbage	90
Leek	4
Red Pepper powder	2
Garlic 2	2
Ginger 1	1
Sugar	1

2.3. Samples

The first, samples of kimchi were prepared transported to the laboratory and were analyzed (Kacem *et al.,* 2005). 25g from samples were homogenized with 225mL sterile sodium citrate solution 2% (w/v), in a Stomacher 400 (Seward Medical, London, UK). Serial decimal dilutions (10^{-2} to 10^{-5}) were made in 0.1% (w/v) peptone solution.(Abdi etal.,2006) Decimal dilution of these samples were mixed with MRS medium (AEB, France) and incubated at 37ºC for 48-72 h under anaerobic conditions.(Lengkey etal., 2009) The numbers of LAB were measured by the plate count on MRS agar (Difco Laboratories, Detroit, USA) mold and yeast were counted on Potato Dextrose agar (Nissui) incubated for 72 h at 30°C. Each LAB colony was purified twice by streaking on MRS agar. Colonies were counted as viable numbers of Microorganisms (colony forming unit (CFU) g^{-1} of kimchi) (Duan *et al.,* 2008)0.1 mL of the diluents were streaked on Nutrient agar for total bacteria counts.

2.4. Determination of pH Values and acidity of the Test Samples

The pH values of each samples were determined at 25C using a pH meter (WTW-Inolab Level 3 Terminal, Weilheim, German). (Yilmaz *et al*., 2010).

2.5. Morphological, physiological, and biochemical tests

Gram stain of LAB and morphological characteristics were determined after 48 h of incubation on MRS agar. Catalase activity and gas production from glucose were determined (Duan *et al.,* 2008). Growth at different temperatures was detected in MRS agar after incubation at 15 and 45^0c. Fermentation of Carbohydrates was determined. The carbohydrates tested were, D (+) galactose, lactose, fructose, maltose, D mannitol, melibiose, D(-) raffinose, sorbitol, D(+) (Merck, Darmstadt, Germany), and glucose (Erdogrul *et al*., 2006). Gas production from glucose were tested in phenol red broth with 2% glucose (Abdi *et al*.,2006).

3. Results and discussion

Results of Microbial analysis and physicochemical tests were given in Table 2 and 3. After series of purification on MRS agar, 443 isolates were found to be Gram-positive, catalase negative.

Table 2
Biochemical and physiological characteristics of LAB isolated from kimchi and comparison with reference LAB strains

code	name	Catalaze test	Geram test	Gas from glucose	Morphology	Growth at		
						45	15	Aerobic conditions
1	*L.fermentum*	-	+	+	bacilli	+	-	+
2	*L.bervis*	-	+	-	bacilli	-	+	+
3	*L.plantarum*	-	+	+	bacilli	+	+	+
4	*L.fructosus*	-	+	-	bacilli	-	+	+
5	*Leu.mesenteroides*	-	+	+	cocci	-	+	+
6	*L.homohiochii*	-	+	-	bacilli	-	+	+

Table 3
sugar test

code	name	sucrose	fructose	gluconate	melibiose	lactose	maltose	raffinose	galactose	mannitol	sorbitol	glucose
1	*L.fermentum*	+	+	+	+	+	+	+	+	-	-	+
2	*L.bervis*	d	+	+	+	d	+	d	d	-	-	+
3	*L.plantarum*	+	+	+	+	+	+	+	+	+	+	+
4	*L.fructosus*	-	+	-	-	-	-	-	-	-	-	+
5	*Leu.mesenteroides*	+	+		d	d	+	d	+	d	-	+
6	*L.homohiochii*	-	+	-	-	-	+	-	-	-	-	+

The results of isolation and identification the standard physiological and biochemical tests were identified that among total isolated 30 isolates of *Leu.mesenteroides*, 50 isolates of *Lactobacillus bervis*, 60 isolates of *Lactobacillus fructosus*, 73 isolates of *Lactobacillus plantarum,* and 90 isolates of *Lactobacillus homohiochii and 140* isolates of *Lactobacillus fermentum.*

Thus, 93.2% of the isolates were *Lactobacillus* and the rest (6.8%) were *leuconostoc.* Frequency of Lactobacillus has been reported in various food components (Torres *et al*, 2006). Images of Identification of isolates are given in Figure 1 to 6.

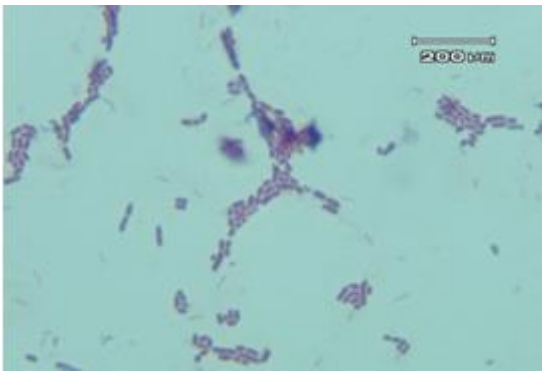

Fig. 1. Isolate *L.fermentum.*

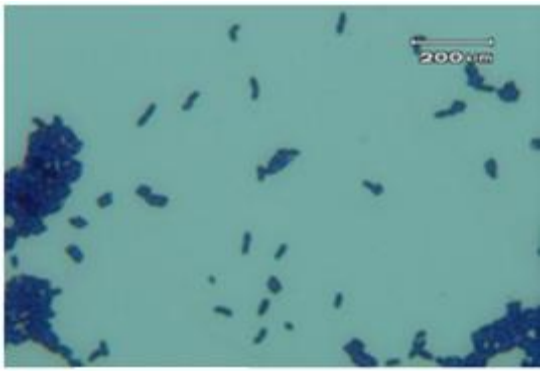

Fig.4. Isolate *L.fructosus.*

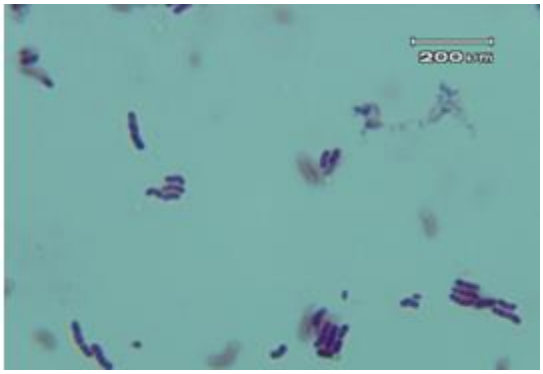

Fig. 2. Isolate *L.bervis.*

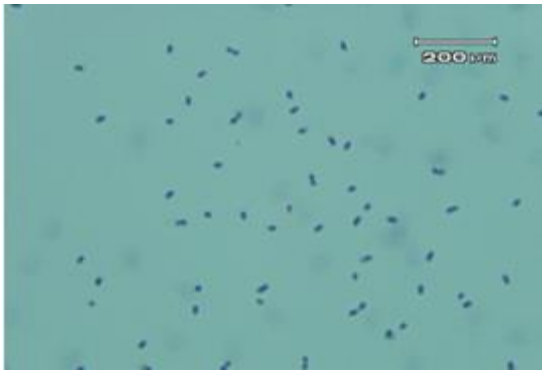

Fig. 5. Isolate *Leu.mesenteroides.*

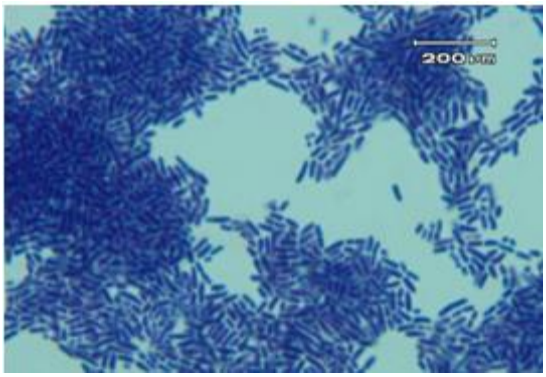

Fig. 3. Isolate *L.plantarum.*

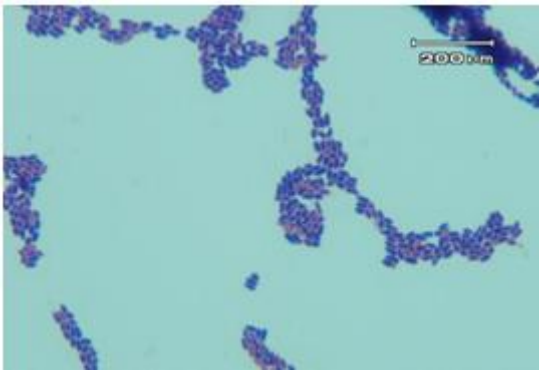

Fig. 6. Isolate *L.homohiochii.*

In the general opinion there are significant similarities between the various generaoflactic acid bacteria in the fermentation of kimchi in Korea and Iran, but there are differences between strains of Lactobacillus, due to differences in the microbial load of used vegetable as and the type and amount of material in the two areas (Figure 7). The LAB in kimchi can be a good source of probiotics. Several cell wall components of LAB have been shown to increase the immune function and to prevent cancer (Lee *et al*., 1996).The results of this study indicate that the kimchi produced in Iran has the probiotic characteristics and the health benefits as well as the Korean samples (Lee *et al.,* 2006).

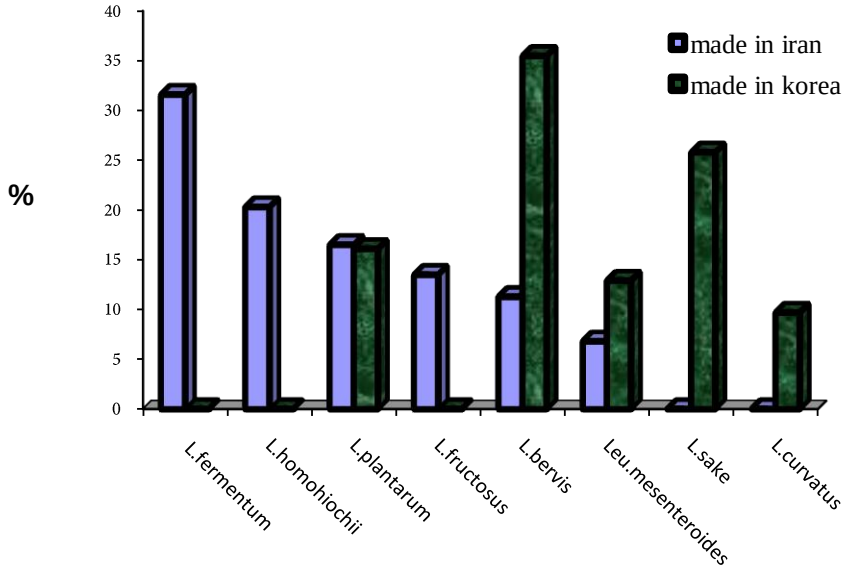

Fig. 7. Distribution of lactic acid bacteria in korean and iranian kimchi.

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