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Antioxidant activity of probiotic lactic acid bacteria isolated from Mongolian airag

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Abstract: This research aimed to determine the antioxidant activity of probiotic lactic acid bacteria isolated from airag. In this study, 42 lactic acid bacteria were isolated from Mongolian airag. All isolates were identified by using morphological, biochemical and physiological methods. The isolated bacteria were studied for antagonistic effects on *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,* 22 strains showed antibacterial activity. When we examined their probiotic properties such as bile acid tolerance and gastric acid tolerance, it is shown thatonly 6 bacterial strains can survive up to 3 hours in a pH 3.0 acid environment and up to 8 hours in 0.3% bile acid environment. Selected probiotic strains were further identified to species by API 50CHL system. Antioxidant activity of probiotic strains were determined by 1,1-diphenyl-2 picrylhydrazyl (DPPH) assay. While the antioxidant activity in cell free supernatant fluctuated between the range of 26.1-38.4%, the antioxidant activity after 72 hours of fermentation in the whey fraction was between 17.23-55.12%.

Keywords: antibacterial activity, acid and bile acid tolerance, DPPH inhibition activity

INTRODUCTION

Lactic acid bacteria (LAB) have been extensively studied for their commercial potential, food preservation and health benefits. They are industrially important microorganisms used worldwide mainly in the dairy industry for manufacturing fermented milk product and cheese.

Lactic acid bacteria and their fermented products are thought to have health promoting probiotic effects in human such as inhibition of pathogenic organism, antimutagenic and reduction of blood cholesterol. Probiotics are defined as *live microorganisms which when administered in adequate amounts confer a health benefit on host* (the Food and Agriculture Organization/ World Health Organization (FAO/WHO) [1]. Most probiotics commercially available today belong to the genera *Lactobacillus* and *Bifidobacterium.* Lactic acid bacteria for use as a probiotic culture must be tolerant to gastric acid and bile, which enables selected strains to survive, grow and perform its therapeutic benefits in the intestinal tract [2, 3].

Antioxidants can serve as preventative agents from different types of disease such as cancer, atherosclerosis and diabetes. Therefore consumption of natural antioxidants through food is helpful for the human health. The fermentation of milk by lactic acid bacteria releases a large number of peptides and amino acids with biological actions, such as angiotensin converting enzyme inhibitory, immune modulatory, opioid and antioxidant activities [4, 5].

There is a wide range of fermented milk products in Mongolia, because of variations in the raw materials. The one of the most common fermented milk product is *airag*, which is mildly alcoholic, sour-tasting fermented drink , made from unpasteurized fresh mare's milk. Fermented products have probiotic effects as they contain live lactic acid bacteria [6]. There are several reports on the LAB strains in the traditional fermented milks of Mongolia [7-9]. However, papers on the probiotic properties of LAB strains isolated from Mongolian traditional fermented milks remain scarce. There is a only few reports have focused on probiotic properties of LAB isolated from Mongolian fermented milk *airag* [10, 11]. So, there is a real necessity to study the biological activity of lactic acid bacteria isolated from fermented milk and to develop technology for making fermented probiotic products. Previously we have isolated bacteriocin producing Enterococcus durans from Mongolian airag, purified bacteriocins and characterized them [12]. This study is part of continuing effort to explore the potentials of our indigenous microbial flora in developing fermented milk products with probiotic effect [13, 14]. Recently, there is no report which has focused on anti-oxidant activity of LAB isolated from

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Mongolian fermented milk. Therefore, we aimed to determine the antioxidant activity of LAB strains isolated from airag.

EXPERIMENTAL

Samples collection: A total of 15 samples of airag were collected from nomads family of Bulgan, Uvurkhangai and Dundgovi provinces. The samples (250 mL) were collected in sterilized bottles and kept under low temperature using an icebox to be brought to the laboratory where they were taken to the procedure for isolation.

Isolation of lactic acid bacteria: All samples (5%, v/v) were propagated twice in sterilized skim milk at 37°C for 16-18 h under anaerobic condition. Samples were serially diluted (10-fold) in peptone water, from 1:10 to $1:10^8$ (v/v) and 1 mL aliquot of the dilution was plated into selective medium MRS (Man, Rogosa and Sharpe, Germany) agar. The plates were incubated at 37°C for 24 h under anaerobic conditions. After incubation, individual colonies were selected and transferred into sterile broth media. The selected colonies were purified by streak plate technique and stored at -20°C in MRS broth with 20% glycerol and kept for further investigation [12].

***Probiotic properties of isolated LAB strains*:** Major selection criteria (antibacterial activity, resistance to low pH and tolerance against bile acid) were chosen for the determination of probiotic properties of isolated LAB strains.

***Antibacterial activity assay*:** The antibacterial activity of cell-free supernatant was determined by well diffusion method as described by Batdorj *et al*. [12]. Cell-free supernatant of isolated bacteria was obtained from 24 hour cultivation at 37°C by centrifugation at 3000 rpm for 15 min. To investigate the antibacterial activity spectra of LAB strains by well diffusion assay, 100 µl culture of one of the test bacteria, grown to the early stationary growth phase in nutrient medium, was added to 20 mL of soft nutrient agar (0.8%, w/v). Wells were made in the lawn of hardened soft agars in Petri dishes. Aliquots (100 µl) of supernatant of overnight cultures (16-18 h) were poured in the wells. The plates were left for 1 h at room temperature in sterile conditions before incubating them to the adequate temperature of growth of the test microorganism. A clear zone of inhibition of at least 1 mm in diameter was recorded as positive.

***Resistance to low Ph*:** Resistance to pH 3.0 is often used *in vitro* assays to determine the resistance to stomach pH. The LAB strains with antibacterial activity (incubated for 16-18 h) were used. Cells were harvested by centrifugation for 10 min at 5000 rpm and 4°C. Cell pellets were suspended in phosphate saline buffer (pH 3.0) and incubated at 37°C. Viable microorganisms were enumerated at the 3 h with pour plate techniques [4].

Tolerance against bile acid. MRS medium containing 0.3% bile acid was inoculated with the LAB strains with antibacterial activity and incubated for 16-18 h. During the incubation for 8 h, viable colonies were enumerated for every hour with pour plate technique and also growth was monitored by absorbance at 560 nm [4].

***Determination of antioxidant activity*:** The DPPH radical scavenging activity was evaluated using the method of Son and Lewis [15]. DPPH radical solution (0.004%, w/v) in 95% ethanol was prepared. A volume of 2 mL of DPPH in ethanol was added to 2 mL of whey fraction, well vortexed and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using UV-Visible spectrophotometer. Ascorbic acid (Sigma, Germany) at a concentration of 0.02 mg/mL was used for comparison. The antioxidant activity of the samples was expressed as percentage of DPPH inhibition activity calculated as:

$$\text{DPPH inhibition activity (\%)} = [(A_{contr}-A_{test})/A_{contr}]\times 100$$

Where:

A_{contr} - absorbance of the control sample (DPPH solution without whey fraction);

A_{test} - the absorbance of test sample (DPPH solution plus whey fraction)

Preparation of cell free supernatant: Sterile MRS broth was inoculated with 1% (v/v) of the overnight grown culture of the six LAB isolates and incubated at 37°C for 18 h. The cell free supernatant was obtained by centrifugation of the overnight grown culture at 10 000 rpm for 5 min at 4°C [16].

Preparation of pre-cultures and fermentations. Each LAB strain was inoculated into 10 mL MRS broth and incubated at 37°C for 24 h. The cultured broths were vortexed and used to inoculate sterilized skimmed milk at a 1% (v/v) concentration, then incubated at 37°C for 24 h to generate pre-cultures. These pre-cultures were used to inoculate fresh pasteurized skimmed milk at 2% (v/v) concentration and fresh pasteurized skimmed milk without LAB served as control. Fermentation was carried out in triplicate at 37°C for 24 to 72 h [16].

***Preparation of whey fraction from fermented milk*:** The whey fraction was prepared as described by Virtanen *et al*. [5] and used immediately after the preparation. Nonhydrolyzed casein was removed. Aliquots (15 mL) were collected from the fermented milk and the pH was adjusted to 4.6 with 1 M HCl. The suspension was centrifuged (10 000 rpm for 20 min at 5°C) and the supernatant was filtered on a 0.45 µm filter.

Identification of isolated LAB strains: Carbohydrate fermentation was determined using API CH system (BioMerieux, France), according to the manufacturer's instruction.

RESULTS AND DISCUSSION.

Isolation of antibacterial LAB strain. We have isolated 42 LAB strains from 15 samples of airag and determined their morphological physiological and biochemical characteristics. All isolated strains were Gram positive and catalase negative, long or short chained rods and coccus. We tested their antibacterial activity by using test microbial strains such as *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Totally twenty two LAB strains have antibacterial activity and only eight of them were shown significant growth inhibiton against indicator strains (Table 1). We have chosen the following LAB strains A-4, A-7, BL-12, BL-13, DU-8, O-9, T-8 and BM8-5 for our further investigation.

Table 1. Antibacterial activity of the LAB strains isolated from airag

LAB strains	Antibacterial activity (Inhibition zone, mm)									
	Cell free supernatant					Neutralized cell free supernatant				
	pH	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	pH	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Lactobacillus paracasei A-4	4.5	6	4	2	3	6.5	5	4	-	3
Lactobacillus plantarum A-7	4	6	6	4	5	6.5	6	5	4	5
Lactobacillus paracasei BL-12	5	6	4	3	3	6.5	5	2	3	1
Lactobacillus plantarum BL-13	4	4	3	6	3	6.5	4	2	5	2
Lactobacillus paracasei DU-8	5	4	4	3	5	6.5	4	3	2	3
Lactobacillus brevis O-9	5	1	4	2	4	6.5	1	3	2	3
Lactococcus lactis T-8	5	6	3	4	4	6.5	5	3	3	3
Lactobacillus lactis BM8-5	4.5	3	1	3	3	6.5	3	-	2	2

The tests were applied two times and the averages of diameters of clear zones were given

***Identification of LAB strains*.** The selected LAB strains were identified with the API 50 CHL Carbohydrate Test Kit (Biomerieux Co., France). The tests were done according to the manufacturer's instruction and the results were interpreted after incubation at 37°C for 48 h. Identification of the LAB strains was done by the interpretation of the fermentation profiles using the computerized database program API WEB software.

As for classification, our study matched the results from different studies that the probiotic lactic acid bacteria that were isolated from the fermented milk are primarily consist of *Lactobacillus plantarum* and *Lactobacillus paracasei* [2, 11]. Takeda *et al.* (2011) reported that 10 homofermented probiotic LAB strains were isolated from Mongolian fermented camel milk, and classified *as L. plantarum* and *L. paracasei*. Khedid *et al.* (2009) characterized LAB isolated from one humped camel milk from Morocco and found that one of the most frequently isolated LAB was *L. paracasei* and *L.plantarum* by 16S rRNA.

***Determination of probiotic properties*.** Resistance to low pH is one of the major selection for probiotic strains. To reach the small intestine, LAB strains have to pass through stomach [2, 6]. For selection of strain resistant to low pH 3.0 was used. The time that takes during digestion in stomach is around 3 h, so LAB strains were tested for resistance to pH 3.0 during 3 h. When we determined eight LAB strains acid tolerance *Lactobacillus paracasei* A-4, *Lactobacillus plantarum* A -7, *Lactobacillus paracasei* BL-12, *Lactobacillus paracasei* DU-8, *Lactobacillus brevis* O-9, *Lactococcus lactis* T-8 were very stable in pH 3.0 which means that LAB strains are able to survive in this pH value. The *Lactobacillus plantarum* BL-13 and *Lactobacillus lactis* BM8-5 were considered acid sensitive and were excluded from further studies.

Table 2. Identification of the LAB strains isolated from airag

Isolate	API kit (50 CHL)	Identification (%)
A-4	*Lactobacillus paracasei*	94%
A-7	*Lactobacillus plantarum*	97.4%
BL-12	*Lactobacillus paracasei*	99.2%
BL-13	*Lactobacillus plantarum*	91.0%
DU-8	*Lactobacillus paracasei*	94.6%
O-9	*Lactobacillus brevis*	97.6%
T-8	*Lactococcus lactis*	95.9%
BM8-5	Not determined	

The pH resistance quality of our LAB strains yielded slightly lower results than reported by other researchers [2, 11]. Maryam *et al.* (2011) reported that LAB strains isolated from Iranian traditional

fermented milk had an acid tolerance from 3.6 to 6.4 Log_{10}CFU/ml. Takeda S *et al.* (2011) also reported that LAB strains isolated from fermented camel milk had an acid tolerance between 7.0 and 8.7 Log_{10}CFU/ml. This may have resulted from the origin of fermented milk that were used to isolate the strains, the different techniques and cultures used for isolation process, and the geographic location from where the fermented milk was collected. Bile acid tolerance is essential for probiotic strains to colonize the small intestine. Selected six LAB strains were tested for their ability to tolerate the bile acid. They all can tolerate the bile salt 0.3% concentration during 8 h. There was substantial variability in resistance to bile acid among the selected LAB strains and all strains tested showed delayed growth, compared to unsupplemented MRS. After the adaptation time (4-6 h) they all showed extensive growth. Similar results were reported in other studies on several species of *Lactobacillus* spp [3, 11].

Determination of antioxidant activity. Lactic acid bacteria are known to have proteolytic activity that hydrolyses protein to produce peptides with bioactivity [4, 5]. The antioxidative potential of LAB has been reported in several studies [16-21].

Firstly we have determined probiotic LAB strain's antioxidant activity in their cell free supernatant as described previously. The selected LAB strains demonstrated the DPPH scavenging activity with inhibition rate in the range 26.1-38.4%. Similar results were reported by Osuntoki *et al.* (2010) that the LAB isolated from African fermented foods scavenged between 6.3 and 33.7% DPPH inhibition activity . The *Lactobacillus brevis* O-9 did not show any activity. The LAB strains *Lactobacillus plantarum* A-7 and *Lactobacillus paracasei* A-4 showed higher inhibition rate in the range of 35.8-38.4%. Secondly we have determined that our 6 probiotic LAB strain's antioxidant activity during the fermentation between 24- 72 h in whey fraction. All LAB strains kept their antioxidant activity in whey fraction and the antioxidant activity increased in most cases during fermentation as shown in Table 4. The measured DPPH inhibition activity of LAB strains in whey fraction was between 3.98-55.12%. Similar result was reported by Maryam *et al.* (2012) that the DPPH inhibition activities varied with LAB strain and ranging from 14.7 to 50.8% for 24 to 72 h fermentation. The strains *Lactobacillus paracasei* A-4, *Lactobacillus plantarum* A-7 and *Lactobacillus brevis* O-9 showed the highest antioxidant activity in the range 45.16-55.12% after 72 h fermentation. Milk fermented with *L. brevis* showed DPPH inhibition values of 54.77%, respectively greater than *L. brevis* (33.7±7.8%) and that reported by Osuntoki and Korie (2010). Also Maryam *et al.* (2012) reported that *L. plantarum* isolated from fruit samples were shown the highest DPPH inhibition activity ranging from 50.8±4.5%. DPPH inhibition activity of whey fraction increased

Table 3. Acid tolerance of the LAB strains isolated from airag

LAB strains	Incubation time, hour			
	0	1	2	3
	Log_{10}CFU/ml			
Lactobacillus paracasei, A-4	3.56	3.33	3.43	3.45
Lactobacillus plantarum, A-7	3.6	3.5	3.45	3.38
Lactobacillus paracasei, BL-12	3.7	3.53	3.4	3.6
Lactobacillus paracasei, DU-8	3.61	3.55	3.58	3.65
Lactobacillus brevis, O-9	3.76	3.65	3.64	3.68
Lactococcus lactis, T-8	3.31	3.4	3.43	3.48

Table 4. Bile acid tolerance of the LAB strains isolated from airag

LAB strains	Medium	Incubation time, hour								
		0	1	2	3	4	5	6	7	8
		Optic density, 560 nm								
Lactobacillus paracasei A-4	MRS	0.06	0.08	0.12	0.37	0.92	1.68	2.25	2.7	2.8
	MRSO-0.3%	0.07	0.05	0.07	0.15	0.18	0.4	0.9	2.0	2.4
Lactobacillus plantarum A-7	MRS	0.06	0.1	0.22	0.78	1.8	2.7	3.0	3.35	3.6
	MRSO-0.3%	0.07	0.05	0.07	0.15	0.39	0.95	2.2	2.9	3.6
Lactobacillus paracasei BL-12	MRS	0.06	0.09	0.17	0.66	1.64	2.7	3.0	3.45	3.6
	MRSO-0.3%	0.08	0.1	0.28	0.75	1.4	2.4	2.8	3.0	3.6
Lactobacillus paracasei DU-8	MRS	0.06	0.09	0.16	0.62	1.62	2.75	3.0	3.45	3.6
	MRSO-0.3%	0.06	0.05	0.11	0.29	0.75	1.4	2.6	3.0	3.0
Lactobacillus brevis ,O-9	MRS	0.06	0.08	0.14	0.54	1.4	2.25	2.85	3.3	3.6
	MRSO-0.3%	0.07	0.06	0.08	0.18	0.38	0.88	1.5	2.8	3.6
Lactococcus lactis, T-8	MRS	0.06	0.06	0.08	0.22	0.58	1.38	2.25	2.85	3.0
	MRSO-0.3%	0.06	0.05	0.06	0.07	0.14	0.3	0.8	2.0	2.9

Table 5. Antioxidant activity of probiotic LAB strains isolated from airag

LAB strains	Antioxidant activity in cell free supernatant, inhibition %	Antioxidant activity in whey fraction, inhibition %		
		Fermentation time, hour		
		24	48	72
Lactobacillus paracasei A-4	35.8	20.89	17.08	45.16
Lactobacillus plantarum A-7	38.4	18.4	21.57	55.12
Lactobacillus paracasei BL-12	26.1	3.98	7.34	19.63
Lactobacillus paracasei DU-8	30.5	10.5	4.02	17.23
Lactobacillus brevis O-9	N/d**	27.79	11.52	54.77
Lactococcus lactis T-8	30.2	11.93	12.65	15.87
Control milk*	10.6	10.6	10.4	10.2
Ascorbic acid	99.5	99.5	99.5	99.5

*Control milk- without LAB strain

**N/d- not determined

with fermentation time. The same results was also reported by other researchers . Virtanen *et al.* (2007) and Maryam *et al.* (2012) reported the DPPH values of milk protein hydrolizate from LAB fermented skim milk increased with the fermentation time. This is the first report on the isolation of lactic acid bacteria with antioxidant activity from Mongolian airag. We assume that probiotic lactic acid bacteria *Lactobacillus paracasei* A-4, *Lactobacillus plantarum* A-7 and *Lactobacillus brevis* O-9 could be used for the preparation of probiotic products with antioxidant activity.

CONCLUSION.

In this study, we have isolated six probiotic LAB strains with anti-oxidant activity, capable of surviving the pH of the stomach and the environment of the intestine, which makes them potential probiotics. The measured anti-oxidant activities differed according to the strain and inhibition in their cell free supernatants varied from 26.1-38.4%. The measured DPPH inhibition activities of LAB strains varied with strains and antioxidant activity ranging from 17.23-55.12% for 72 h fermentation.

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REFERENCES

1. World health organization (WHO) 2002. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria , a joint FAO/WHO expert consultation. http://www.who.int/foodsafety/publicationa/ fs_management/probiotics/en/index.html
2. Maryam T.E., Arthur C.O., Mohamad A.H., *et al* (2011) Traditional Iranian dairy products: A source of potential probiotic Lactobacilli. *Afr. J. Microbiol. Res.*, **5**, 20-27
3. Rasha H., Walla S., Abdel-all., Mostafa G., *et al.,* (2012) Characterization of Lactic Acid Bacteria isolated from Dairy products in Egypt as a probiotic. *J. Life Sci.*, **9**, 2924-2933.
4. Anne P., Virtanen T., Akkanen S., *et al.* (2006) Anti -oxidative peptides derived from milk proteins. *Int Dairy J*., **16**, 1306-1314
5. Virtanen T., Pihlanto A., Akkanen S. *et al.* (2007) Development of anti-oxidant activity in milk whey during fermentation with lactic acid bacteria. *J. Appl. Microbiol.,* **102**, 106-115
6. Uchida K., Hirata M., Motoshima H., et al (2007) Micribiota of *airag, tarag* and other kinds of fermented dairy products from nomad in Mongolia. *J. Anim. Sci*., **78**, 650-658
7. Yu j., Wang W.H., Menghe B.L., *et al.* (2011) Diversity of lactic acid bacteria associated with traditional fermented dairy products in Mongolia. *J. Dairy Sci.,* **94**, 3229-3241
8. Watanabe K., Fujimoto J., Demberel S., *et al.* (2008) Diversity of lactic acid bacteria and yeasts in Airag and Tarag, traditional fermented milk products of Mongolia. *World J. Microbiol. Biotechnol*., **24**, 1313-1325
9. Sun H.Z., Lui W.J., Zhang J.C., *et al.* (2010) Identification and Characterization of the Dominant Lactic acid Bacteria from Traditional Fermented Milk in Mongolia. *J. Folia Microbiol.,* **55**, (3), 270-276
10. Watanabe K., Fujimoto J., Demberel S., *et al.* (2009) Bifidobacterium mongoliense sp.nov., from airag, a traditional fermented mare's milk product from Mongolia. *J. Systematic and Evolutionary Microbiol*., **59**, 1535-1540
11. Takeda S., Tsend-Ayush Ch., Dashnyam B., *et al.* (2011) The investigation of probiotic potential of lactic acid bacteria isolated from traditional Mongolian dairy products. *J. Animal Science,* **82,** 571-579
12. Batdorj B., Dalgalarrondo M., Choiset Y., *et al.* (2006) Purification and characterization of two bacteriocins produced by lactic acid bacteria

isolated from Mongolian airag. *J. Appl. Microbiol*., **101**, 837-848

13. Budragchaa D., Batjargal B., Sukhdolgor J. (2009) Possibility to make a cheese by using local lactic acid bacteria isolated from camel khoormog. *Food study-2009: Proceeding of national scientific conference.* Ulaanbaatar, 104-108 (in Mongolian)
14. Munkhtsetseg B., Margad-Erdene M., Batjargal B. (2009) Isolation of Lactic Acid Bacteria with High Biological Activity from Local Fermented Dairy Products. *Mongolian Journal of Biological Sciences*, **7**, 61-68
15. Son S., Lewis B.A. (2002) Free radical scavenging and anti-oxidative activity of caffeic acid amide and ester analogues: Structure-activity relationship. *J. Agric. Food Chem.,* **50**, 468-472
16. Abd El-Moneim, Afify M.R., Ramy M.Romeilah., Shaimaa I.Msultan *et al*. (2012) Antioxidant activity and Biological evaluations of Probiotic bacteria strains. *J. Acad. Res.,* **4**, 131-139.
17. Kullisaar T., Songisepp E., Mikelsaar M. *et al*. (2003) Anti-oxidative probiotic fermented goat's milk decreases oxidative stress-mediated atherogenity in human subjects. *Br. J. Nutr.,* **90**, 449-456
18. Kaizu H., Sasaki M., Nakajima H. et al. (1993) Effect of anti-oxidative lactic acid bacteria on rats fed a diet deficient in vitamin E. *J Dairy Sci.,* **76**, 2493-2499
19. Akinniyi O., Ifeoma K. (2010) Antioxidant activity of whey from fermented with Lactobacillus species isolated from Nigerian fermented foods. *J. Food Technol. Biotechnol*., **48**, 505-511
20. Maryam A.S.Abubakr, Zaiton Hassan, Mohamed Muftah A., *et al.* (2012) Antioxidant activity of lactic acid bacteria fermented skim milk as determined by DPPH and ferrous chelating activity. *Afr. J. Microbiol. Res.,* **34**, 6358-6364.