УДК 637.146.32

PROBIOTIC CHARACTERISTICS OF LACTIC ACID BACTERIA ISOLATED FROM MONGOLIAN FERMENTED DAIRY PRODUCT AIRAG

E. Uugantsetseg [1], B. Batjargal[2], B. Munkhtsetseg[3]

(Department of Chemistry, School of Natural Sciences, Mongolian State University of Agriculture, Department of Biochemistry and Bioorganic Chemistry, School of Biology and Biotechnology, National University of Mongolia)

This research aimed to isolate lactic acid bacteria with a significant probiotic character from Mongolian fermented dairy product airag. In this study, 37 lactic acid bacteria were isolated from Mongolian airag. All isolates were identified by using morphological, biochemical and physiological methods. Among the isolates, 25 strains have antimicrobial activity. Probiotic properties of isolates were investigated. The isolated bacteria were studied for antagonistic effects on Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aerues, Escherichia coli and Micrococcus luteus. We determined that among the isolates the strain S14, S22, S25, S28 and S32 were shown the highest antimicrobial activity. When we examined their probiotic properties such as bile acid tolerance and gastric acid tolerance, it is shown that these two bacterial strains can last up to 24 hours in a pH 2.5 artificial stomach acid, and up to 6-8 hours in a 0.3%

[1]Э.Уганцэцэг - Монгольский Сельскохозяйственный Университет, Филиал скотоводства и биотехнологии, Кафедра химии
E. Uugantsetseg - Department of Chemistry, School of Natural Sciences, Mongolian State University of Agriculture
[2]Б. Батжаргал - Монгольский Государственный Университет, Филиал наука, Кафедра биоорганической химии и биохимии
B. Batjargal - Department of Biochemistry and Bioorganic Chemistry, School of Biology and Biotechnology, National University of Mongolia
[3]Б. Мөнхцэцэг - Монгольский Государственный Университет, Филиал наука, Кафедра биоорганической химии и биохимии
B. Munkhtsetseg - Department of Biochemistry and Bioorganic Chemistry, School of Biology and Biotechnology, National University of Mongolia

bile environment. This proves that these strains can be used as culture for producing probiotic dairy products. When these two strains are examined by the API 50 CH analysis, it is determined that strain S28 is Lactobacillus rhamnosus and the S32 is Lactobacillus paracasei.

***Key words**: antimicrobial activity, acid and bile acid tolerance*

INTRODUCTION

Lactic acid bacteria 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 food 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 abequate amounts confer a health benefit on host'' (the Food and Agriculture Organization/ World Health Organization (FAO/WHO). 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 acid and bile, which enables selected strains to survive, grow and perform its therapeutic benefits in the intestinal tract.

There is a wide range of fermented milk products in Mongolia, because of variations in the raw materials. The most common fermented milk product is airag, which traditionally made from mare's milk. Another kind of fermented milk is khoormog, which is prepared from camel milk.

Lactic acid bacteria play in vital role for fermentation of Mongolian traditional dairy products. Currently, most of the studies on Mongolian dairy product's microbiology have been conducted only in systematic of microorganisms. So, there is a real necessity to study the biological activity of lactic acid bacteria and to develop technology for making probiotic products.

This study is a part of continuing effort to explore the potentials of our indigenous microbial flora in developing fermented milk products with probiotic effect. (Batdorj et al., 2006; 2007; Budragchaa et al., 2009)

MATERIAL AND METHODS

Samples collection. A total of 15 samples of airag were collected from nomads family of Bulgan provinces in Mongolia. 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. 2. Isolation of Lactic acid bacteria. All samples (10%, v/v) were propagated twice in skim and sterilized milk at 37^0c for 16-18h under anaerobic condition. Samples were diluted to 10^{-8} using sterile peptone water and 1 ml aliquot of the dilution was plated into selective medium MRS (Man, Rogosa and Sharpe) agar. The plates were incubated at 37^0c 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.

The isolates were examined according to their colony morphology, catalase reaction and Gram reaction survival at different temperature and tolerance to NaCI concentrations also methyl red. Gram positive and catalase negative colonies were taken as lactic acid bacteria and stored at -20^0c in MRS (Man, Rogosa Sharpe) broth (Carl Roth, Germany) with 20% glycerol and kept for further investigation.

Before use, the lactic acid bacterial strains were propagated twice in the appropriate broth overnight. Agar media were prepared by addition of 1.5 % (w/v) agar (Carl Roth, Germany) to the medium overlay agar and agar well base contained 0.8% (w/v) of agar. The bacterial strains which were used to evaluation for antimicrobial activities are Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa were cultivated in Nutrient Agar medium (NA).

Probiotic properties of isolated LAB strains. Major selection criteria (resistance to low pH, tolerance against bile acid and

the antimicrobial activity) were choosen for the determination of probiotic properties of isolates.

Resistance to Low pH. Resistance to pH 3 is often used *in vitro* assays to determine the resistance to stomach pH. Active cultures (incubated for 16-18 hrs) were used. Cells were harvested by centrifugation for 10 min at 5000 rpm and 4^0c. Cell pellets were suspended in phosphate saline buffer (pH 3) and incubated at 37^0c. Viable microorganisms were enumerated at the 3 hours with pour plate techniques.

Tolerance against bile acid. The mean intestinal bile concentration is believed to be 0.3% (w/v) and the staying time of food in small intestine is suggested to be 4 hrs, the experiment was applied at this concentration of bile for 4 hrs. MRS medium containing 0.3% bile was inoculated with active cultures and incubated for 16-18hrs. during the incubation for 4 hrs, viable colonies were enumerated for every hour with pour plate technique and also growth was monitored by absorbance at 560 nm.

Antimicrobial activity assay. The antimicrobial activity of cell-free supernatant was determined by well diffusion method as described by Batdorj *et al*., (2006). Cell-free supernatant was obtained 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.

5. Identification of isolated LAB strains.

Results. Isolation of antimicrobial strain. We have isolated thirty seven 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 antimicrobial activity by using test microbial strains such as *Escherichia coli and Enterococcus faecalis.*

Totally 25 isolates have a significant growth inhibiton against indicator strains and we have choosen 5 strains which were shown highest result for our further investigation.

Table 1. Antimicrobial activity of isolated bacteria

Isolate	Antimicrobial activity (Inhibition zone,mm)					
	Cell free supernatant					
	pH	Ps. aeruginosa	Ent. faecalis	Staphylococcus aereus	E.coli	Micrococcus luteus
S14	4.5	3	4	2	2	1
S22	4.5	3	3	2	1	5
S25	5.0	2	3	1	3	2
S28	4.0	3	1	3	4	1
S32	4.0	2	5	2	3	5

Resistance to low pH is one of the major selection for probiotic strains. When we determined our 5 strains gastric acid tolerance, the isolates S28, S32 were very stable in pH 2.5

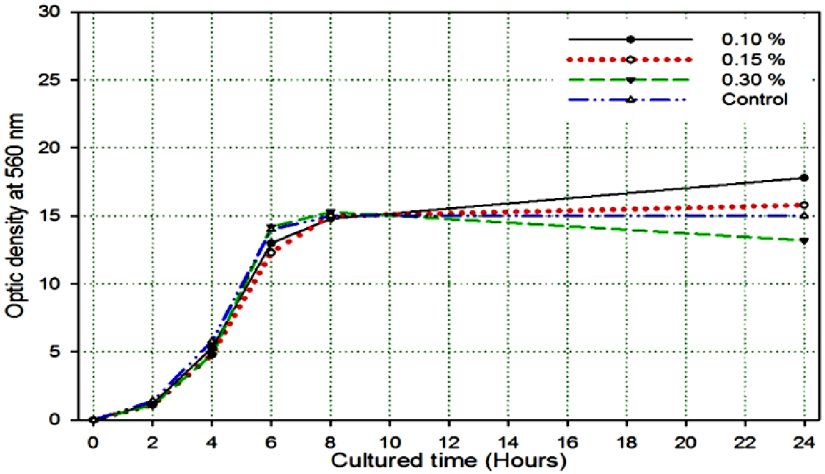
Table 2. Gastric acid tolerance of the selected strains

Isolate	pH	Log cfu/ml					
		0	1	2	3	4	24
S28	2.5	9.3	8.5	7.5	5.7	4.6	3.9
S32	2.5	9.3	8.9	7.6	6.3	5.6	4.4

Bile acid tolerance is essential for probiotic strains to colonize the small intestine. S28, S32 strains were tested for bile acid tolerance.

These 2 strains can tolerate the bile salt 0.3% concentration during 8 hours.

The S32 strains results were shown in figure 1.



When these two strains are examined by the API 50 CH (bio-Merieux, France) analysis, it is determined that strain S28 is Lactobacillus rhamnosus and the S32 is Lactobacillus paracasei.

Discussion. Recently the most of the studies on Mongolian dairy product's microbiology have been conducted only in systematic of microorganisms (Damdinsuren *et al*., 2008; Koichi *et al*., 2008; Watanabe *et al*., 2008). There is real necessity to study the biological activity of lactic acid bacteria and to develop technology for making probiotic products. Previously we have isolated bacteriocin producing *Entererococcus durans* from Mongolian airag, purified bacteriocins and characterized them (Batdorj *et al.,* 2006). It is tested and approved that this bacteria shows a high influence against the food-born pathogens and pathogenic bacteria.

References

1. Baldorj, R. & Namsrai, Ts. 1980. *Mongolian Airag*. State press, Ulaanbaatar. pp. 13-25. [in Mongolian]

2. Batdorj et all., 2006. Purification and characterization of two bacteriocins produced by lactic acid bacteria isolated from Mongolian airag. *J. Appl. Microbiol*., 101: 837-848.

3. Batdorj et all., 2007. Inhibition activity on food-born pathogens by hydrogen peroxide producing Lactobacillus delbrueckii subsp. lactis T31, isolated from Mongolian yoghurt. *J. Appl. Microbiol*., 103: 584 -593.

4. Batdorj. E "The study of bacteriocins by lactic acid bacteria isolated from Mongolian airag" dissertation, 2006, Nance, France

5. Fuller, R., Probiotics in man and animals. Appl. Bacteriol., 66: 365-378

6. Havenaar, R. and J.Huis in't Velt, 1992. Probiotics: A general view. The lactic acid bacteria in health and disease. London Elsevier Appl.Sci., pp: 209-224

7. Salminen, S., A.C. Ouwehand and Isolauri, 1998. Clinical applications of probiotic bacteria. Inter. Dairy J., 8: 563-572

8. Salminen, S. and A. von Wright, 1993 Lactic acid bacteria. New York, Basel, Hong Kong.

9. Usman. P., 2003. Bile and acid tolerance of lactic acid isolated from Dadih. Asian-Australian J. Animal. Sci., 16: 1680-1685

10. Rashid, M.H., K. Togo. And T. Miyamoto. 2007. Identification and characterization of lactic acid bacteria isolated from traditional fermented milk Dahi in Bangladesh. World J. Microbiol. Biotechnol., 23: 125-133

11. Itoh, T., 1992. Functional benefits from lactic acid bacteria used in cultured milk. Anim.Sci.Technol., 63: 1276-1289

12. Gohran. M., 1994. Lactobaciillus with the ability of colonization and establishing in the intestinal tracts. Japan Patent. 6: 501-624