ANTIGENOTOXIC ACTION OF "NARINE" LACTOBACILLI IN RAT COLON CELLS *IN VITRO*

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ДНК–ПРОТЕКТОРНОЕ ДЕЙСТВИЕ ЛАКТОБАЦИЛЛ "НАРИНЕ" НА КЛЕТКИ КИШЕЧНИКА КРЫС *IN VITRO*

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Possible antigenotoxic action of lactobacilli "Narine" (LB, *Lactobacillus acidophilus*, strain 317/402), widely used for milk fermentation for babies' food in Armenia was studied *in vitro* using the single cell gel electrophoresis technique (the comet assay). Whole 24 h LB culture, supernatant of this culture and pellet, supplemented with fresh growth medium were incubated with rat colon cells and potent carcinogen N-methyl-N′-nitro-N-nitrosoguanidine. The results have shown that only pellet of lactobacilli culture supplemented with fresh growth medium decreased significantly the length of DNA migration (DNA damage).
***Key Words*: the comet assay; lactobacilli "Narine"; N-methyl-N′-nitro-N-nitrosoguanidine.**

С помощью метода ДНК-комет изучали возможный ДНК-протекторный эффект лактобацилл "Нарине" (*Lactobacillus acidophilus*, штамм 317/402), которые широко используют в Армении для ферментации молока, предназначенного для детского питания. Суточную культуру лактобацилл, ее супернатант и осадок, полученный после центрифугирования и разведенный свежей средой, инкубировали с клетками кишечника крыс и канцерогеном N-метил-N′-нитро-N-нитрозогуанидином. В результате экспериментов установлено, что лактобациллы с добавлением свежей среды вызывали достоверное снижение повреждений ДНК, индуцированных канцерогеном.
***Ключевые слова*: метод ДНК-комет; лактобациллы "Нарине"; N-метил-N′-нитро-N-нитрозогуанидин.**

During last years possible anticarcinogenic, antimutagenic and antitumor properties of lactobacilli (LB) were discussed [1–4]. An inverse relationship has been demonstrated between the frequencies of consumption of fermented milk products with live LB and breast cancer in women [5]. The process of induction of malignant tumors involves mutations in oncogenes, tumor suppressor genes and for DNA-repair genes [6]. Protective effects of some strains of LB on chemical mutagenesis and genotoxicity *in vivo* (chromosomal aberrations and micronuclei in bone marrow cells of rodents, the comet assay in gastric and colon cells of rats) and *in vitro* (the comet assay and the Ames assay) have been found [3, 7–9]. Recently in our laboratory has been shown that milk fermented with *Lactobacillus acidophilus*, strain 317/402 (called "Narine" in Armenia and widely used for babies' food) — LBN — significantly reduced the frequencies of chromosomal aberrations in bone marrow cells of rats induced by cyclophosphamide and thiotepa [10].

The aim of the present work was to study the influence of LBN on DNA damaging activity of N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), strong rodent colon carcinogen, on rat colon cells because many experimental studies evidenced that LB in general can prevent colon cancer [1, 2].

LBN were inoculated into "de Man, Rogosa, Sharpe" (MRS, Merck, Germany) broth and incubated at 36°C for 24 h to obtain stationary phase cells. In experiments the approach proposed by Pool-Zobel [11] was used — the investigation of antigenotoxic action of whole LBN 24 h culture (10^9 cells/ml), it's supernatant, and pellet of the culture supplemented with fresh MRS medium. Supernatant and pellet of the culture were obtained after centrifugation of whole LBN culture for 10 min at 10,000 rpm. Volumes of all used culture and culture fractions were 4.5 ml. In all mentioned tubes MNNG (Merck, Germany) was added at a volume of 500 µl (concentration 1 µg/ml). As a negative (solvent) control only MRS medium was used whereas positive control consisted of MRS + MNNG. All tubes were incubated for 1 h at 37°C. Three male Sprague-Dowley rats (280–320 g) from animal room of the Institute for Nutritional Physiology, Karsruhe, Germany were sacrificed by asphyxiation under CO_2. Five cm of colon were isolated, flushed with buffer (10 min) and filled with digestive solution containing protease (type XI, Sigma, Germany, 50 U/ml). Colon was incubated at 37°C for 30 min. The isolated cells were centrifuged (8 min, 500 rpm), resuspended in RPMI 1640 (Merck, Germany). Colon cells were studied for viability using trypan blue exclusion both before and after incubation with LBN with/without carcinogen. In all three experiments viability of colon cells was about 90% in all cases. Then the cells were added to all tubes (100 µl, $2 \cdot 10^5$ cells/ml) which

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Abbreviations used: LB –lactobacilli; LBN — *Lactobacillus acidophilus*, strain 317/402 (''Narine''); MNNG — N-methyl-N′-nitro-N-nitrosoguanidine.

were incubated for 30 min at 37°C. The single cell gel electrophoresis method (the comet assay) was used to study possible protective effects of LBN on DNA damage induced by carcinogen. The cells were washed by centrifugation, and 10 µl aliquots containing $2 \cdot 10^5$ cells of the colon were embedded into 65 µl of low–melting agarose and distributed onto microscopic slides coated with one layer of normal agarose and one layer of low–melting agarose. After they hardened, the slides were submerged in a lysis solution, subjected to electrophoresis, and finally stained with ethidium bromide, as described elsewhere [7, 11, 12]. For DNA damage evaluation, comet length was measured from 101 cells (2 slides per every variant; two separate cultures) using computerized image analyzer (Perceptive Instruments, UK). Percent of cells with comet length < 35 µm (undamaged cells), 35–69 µm, 70–110 µm, > 110 µm was counted. The total score of comets was also evaluated in arbitrary units based on the DNA damage (comet length). Student's *t*–test was used to study the differences between the experimental groups.

The results of our experiments are presented in Table. Analysis of the data showed that the length of the comets (the degree of DNA migration) increased significantly under the action of carcinogen (by 97.35%). 24 h whole culture of LBN and pellet of LBN culture supplemented with fresh medium decreased significantly DNA damage induced by carcinogen by 11.6% and 31.0%, respectively. Biological significance of reduced length of comets (DNA damage) induced by whole culture of LBN is questionable because DNA damage presented in arbitrary units and the percent of undamaged cells were not differed significantly compared with positive control. Pellet of culture supplemented with fresh medium decreased significantly the length of comets expressed in arbitrary units (by 25.4%) and increased the percent of undamaged cells (from 16.0% to 54.5%, 3.4–fold increase).

So, only fresh culture of LBN decreased significantly DNA damage induced by strong rodent gastric and colon carcinogen MNNG. Our results confirmed the data obtained by research group of Prof. B. Pool–Zobel [11, 12] that only pelleted strains of lactobacilli (*L. acidophilus, L. gasseri, L. confusus, S. thermophilis, B. breve, B. longum*), supplemented with fresh medium were antigenotoxic *in vitro*. It was proposed that short lived products from culture with viable multiplying lactobacilli are active antigenotoxic agents.

LBN did not induce DNA damage. Recently we did not observed clastogenic effect of LBN [10]. It is noteworthy that in mice an intraperitoneal injection of *L. delbrueckii* induced significantly high frequency of chromosomal aberrations in bone marrow cells, micronucleated erythrocytes and sperm with abnormal head morphology [13].

Table. The influence of lactobacilli "Narine" on DNA damage in rat colon cells (mean ± SEM)

Experimental groups	Length of comets, µm	Arbitrary units	Undamaged cells, %
Negative control	30.95 ± 0.35	23.7 ± 3.0	81.8 ± 2.5
Positive control	61.08 ± 0.73	110.8 ± 11.2	16.0 ± 4.8
Whole culture + MNNG	53.98 ± 0.56**	101.5 ± 8.2	23.7 ± 2.7
Supernatant + MNNG	59.77 ± 0.51	103.2 ± 12.9	20.0 ± 3.2
Pellet + MNNG	42.12 ± 0.42***	82.7 ± 7.6*	54.5 ± 5.5**

The difference between positive control is significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

In conclusion, LBN widely used In Armenia as babies' food, can prevent genotoxic action of strong carcinogen MNNG in rat colon cells *in vitro*. These data confirm our results obtained in *in vivo* system (decrease of chromosomal aberrations level induced by some carcinogens in bone marrow cells of rats) [10] and research findings of Pool–Zobel et al [11, 12] that pellets of LB culture supplemented with fresh medium possessed the most high antigenotoxic activity. Further investigation of antigenotoxic/anticarcinogenic activity of LBN are certainly warranted.

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