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STUDY OF BIOCOMPATIBILITY AND ANTITUMOR ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM THE HUMAN GASTROINTESTINAL TRACT

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Abstract

Objective: to study the biocompatibility of 17 strains of lactic acid bacteria previously isolated from the gastrointestinal tract of healthy people and people with oncological diseases.

Methods: Lactic acid bacteria obtained from the digestive tract of healthy people and people with oncological diseases sick people from different age groups served as the basis of composing symbiotic consortia with a view to expand their effect. In vitro studies proved their inhibitory effect against lymphoma cell cultures, prostate cancer, breast cancer, hepatocellular carcinoma, brain cancer and pancreas cancer.

Results: The paper established that biocompatibility of all group strains is characteristic of the Bifidobacterium breve and Lactobacillus casei strains, conditional biocompatibility can be identified in Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus acidophilus and Lactobacillus paracasei, respectively for the first and second groups. Biological incompatibility was observed in combinations: Bifidobacterium bifidum + Micrococcus spp. ; Lactobacillus salivarius + Micrococcus spp.

Conclusions: Study of antitumor properties of lactobacilli consortia composed of 17 previously selected probiotic strains demonstrate high antitumor activity of these consortia to cancer cell lines of Burkitts lymphoma (BRL2),

human prostate cancer DU 145, human breast cancer MDAMB-231 and MCF7, hepatocellular carcinoma HepG2, brain cancer U-87, human pancreatic cancer PANC-1.

Keywords: Anti-cancer, Probiotic strains, Cancer patients, Bioactive microbial metabolites, Gastrointestinal tract.

Introduction

Cancer is one of the predominant causes of death, second only to cardiovascular disease. Depending on the stage of the cancer, treatment typically involves surgical intervention in combination with radiation or chemotherapy, side effects of which cause damage to human health that is not smaller than that caused by the disease itself. There is a vital need for alternative methods and new scientific approaches, which can be created by combining complementary technologies: high-throughput screening methods of bioinformatics, genomics, combinatorial biosynthesis, etc. Many countries actively research this area. One of the fields that deserves the attention in terms of both treatment and prevention of cancer is immunotherapy and methods that use natural biologically active substances (including in chemotherapy) that have significantly fewer side effects. Plants and microorganisms are regarded as sources of such materials (Forster et al., 2013; Keijer, Bekkenkamp-Grovenstein, Venema, & Dommels, 2011; Neumann, Fujimori, & Walsh, 2008; Petrova et al., 2008; Umezawa, 2008).

Among the latter, special attention is drawn by lactic acid bacteria, usually of two genera – *Lactobacillus* and *Bifidobacterium*, as well as by *Saccharomyces* and *Enterococcus* (Nami et al., 2014; Thirabunyanon, Boonprasom, & Niamsup, 2009), which proved to be effective in the prevention and treatment of a wide range of diseases (de Moreno de LeBlanc, Matar, & Perdigon, 2007; Maragkoudakis et al., 2006; Rafter, 2003; Saikali, Picard, Freitas, & Holt, 2004; Zhu, Michelle Luo, Jobin, & Young, 2011).

Ahmadi et al. studied the antimutagenic and anticarcinogenic effect of 25 strains of lactic acid bacteria isolated from Tarkhans. During researches four isolates of *Lactobacillus spp.* were identified (Ahmadi et al., 2014). The increased antimutagenic activity against sodium azide in cell suspensions of four strains compared to supernatants was observed. The maximum inhibition percentage of cell suspensions was 60.38%. Antimutagens of these strains were increased in the presence of rat S9. The four selected strains were identified on the basis of 16S rDNA-sequencing. The strains of *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus brevis*, showing high antimutagenic and anticarcinogenic activity were isolated from Tarkhans. 4 strains of the 25 strains of lactic acid bacteria with elevated antimutagenic strains were selected as new probiotic strains (Commane, Hughes, Shortt, & Rowland, 2005).

Escamilla et al. studied the ability of the cell-free supernatants (CFS) of probiotic strains of *Lactobacillus casei* and *Lactobacillus rhamnosus* GG to inhibit colon cancer cells *in vitro*. HCT-116 cells were treated with CFS *L. casei*, *L. rhamnosus*, or *Bacteroides thetaiotaomicron*; or inoculated with the bacterial medium. CFS processing of both types of *Lactobacillus* sp. decreased the invasion of colon cancer cells, whereas the treatment of CFS *B. thetaiotaomicron* had no effect on this process. CFS of both types of *Lactobacillus* sp. reduced level of matrix metalloproteinase-9 (MMP-9), and increased protein levels of zona occludens-1 (ZO-1). CFS *L. rhamnosus* also reduced the activity of MMP-9. In order to explain observed phenomena CFS *Lactobacillus* sp. were fractionated in certain ranges of molecular weights, and cell invasion was evaluated. Fractionation revealed that inhibitory activity is characterized mainly for fractions of > 100 kDa and 50-100 kDa. This suggests that the inhibitory compounds can be macromolecules such as proteins, nucleic acids or polysaccharides (Escamilla, Lane, & Maitin, 2012).

Wan et al. studied the effect of supernatants derived from the fermentation of *Lactobacillus delbrueckii* (LBF) on colon cancer. The results indicate that the proliferation of colon carcinoma cells SW620, treated with a solution of LBF, stops and accumulates in phase G1, the nature of the accumulation is dependent on the concentration. The solution of LBF effectively induces apoptosis through the inner kapkaza-3-dependent pathway to the corresponding decrease in expression of Bcl-2. The activity of matrix metalloproteinase-9, which is associated with invasion of colon cancer cells, was also reduced in cells treated with LBF. These results indicate the antitumor effect of LBF *in vitro* and contribute to the development of new therapeutic agents for the treatment of colon cancer (Wan et al., 2014).

Orlando et al. investigated the antiproliferative and proapoptotic properties of *Lactobacillus paracasei* IMPC2.1 and *Lactobacillus rhamnosus* GG strains with respect to cell lines of stomach cancer and HGC-27 colon DLD-1. It is found that cells of gastric cancer, and colon cancer cells were sensitive to growth inhibition and apoptosis induced by *L. Paracasei* IMPC2.1 strains and *L. Rhamnosus* GG. These data offer to use these strains for creating functional foods for the prevention and treatment of cancer (Orlando et al., 2012).

Hwang et al. studied gastric cancer cell apoptosis induced by extracts of *Lactobacillus casei* (LBX) through inhibition of NF- κ B and mTOR-Mediated Signaling. Proliferation and cell death of KATO3 gastric cancer after treatment with LBX for different durations and different doses was investigated. LBX inhibited the growth of gastric cancer cells and induced apoptosis of the promoter inactivation of NF- κ B. Apoptosis was induced by LBX, but not directly connected to the inner mitochondrial way. Immuno-blot analysis revealed that the expression of LBX reduce NF- κ B

and IκB. Reduced levels of NF-κB result in a decrease of mTOR phosphorylation signaling components such as PI3K, Akt, and p70 S6 kinase. The results obtained indicate that LBX induces apoptosis of gastric cancer cells by inhibiting NF-κB and mTOR-mediated signaling (Hwang et al., 2013).

The inhibition of oral cancer induced by 4-nitroquinoline-1-oxide *Lactobacillus salivarius* REN strain was studied (Zhang et al., 2013). New probiotic *Lactobacillus salivarius* REN (*L. salivarius* REN) isolated in China and exhibits a wide range of anti-genotoxicity. In order to study antitumor activity of *L. salivarius* REN *in vivo* 4-nitroquinoline-1-oxide (4NQO) model of induced oral cancer was used. As a result, oral treatment by probiotic *L. salivarius* REN or its metabolites effectively suppresses 4-nitroquinoline-1-oxide-induced oral carcinogenesis on initial and subsequent stages, the degree of inhibition depends on the dose of the probiotic. Significant reduction of neoplasms (65% -0%) was found in the diet of rats, which included high doses of *L. salivarius* REN [$5 \cdot 10^{10}$ CFU/kg body weight (bw)/d]. *In vivo* studies have shown that probiotics inhibit 4NQO-induced oral cancer, protecting DNA from oxidative damage and reduces the expression of COX-2. Treatment of *L. salivarius* REN significantly reduced expression of proliferating cell nuclear antigen (PCNA) and induced apoptosis in a dose dependent manner. The results indicate that probiotics may operate as potential agents for the treatment of oral cancer (Burns & Rowland, 2000).

Prisciandaro et al. investigated changes preventing activation of caspase-3 and 7 and the transepithelial electrical stability under the action of the probiotic factors in models of 5-fluorouracil-induced destruction of epithelial cells. The authors studied the ability of supernatants (SN) *Escherichia coli* Nissle 1917 (EcN) and *Lactobacillus rhamnosus* GG (LGG) to prevent 5-FU-induced destruction of epithelial cells for 5 days. IEC-6 cells were treated daily with 1 ml of PBS (control), de Man Rogosa Sharpe (MRS) broth, tryptone soy broth (TSB), LGG SN, or EcN SN. Except for the control all study groups were treated with 5-FU (5 μM) for 24 hours on the third day. Transepithelial electrical resistance (TEER) was measured at 3, 4 and 5 days, while 3 and 7 activation of caspase at 4 and 5 of apoptosis evaluation. Pretreatment of LGG SN has increased TEER ($p < 0.05$) as compared to control on the third day. Treatment with 5-FU reduced TEER in comparison to untreated cells on the 4th or 5th day. Pretreatment of MRS, LGG SN, TSB, and partially prevent the EcN SN decrease in TEER, induced by 5-FU on day 4, whereas EcN SN also improved TEER in comparison to the control. These differences were also observed on day 5 together with a significant improvement of TEER in cells treated with LGG EcN SN and in comparison with healthy controls. 5-FU has increased activity of caspase by 4 or day 5 in comparison to the control. On day 4 all cells treated with MRS, TSB, LGG SN, or EcN SN, showed a reduction of caspase activity compared with 5-FU controls, whereas SN both

groups had a significantly lower caspase activity than the controls. Caspase activity in cells pretreated with MRS, LGG SN, and EcN SN, is also reduced at day 5 compared to 5-FU controls. Thus, pretreatment by SN probiotic can prevent or to inhibit apoptosis of enterocytes (Prisciandaro et al., 2012).

In the present study, five symbiotic consortia composed of strains of lactic acid bacteria were isolated from the human gastro-intestinal tract in order to identify the one with the strongest antitumor activity.

Experimental Section

Lactic acid bacteria isolates, media and cultivation conditions

Seventeen strains of lactic acid bacteria, indicated as K1 – *Bifidobacterium bifidum*, K2 – *Bifidobacterium breve*, K3 – *Bifidobacterium longum*, K4 – *Bifidobacterium adolescentis*, K5 – *Lactobacillus fermentum*, K6 – *Lactobacillus plantarum*, K7 – *Lactobacillus acidophilus*, K8 – *Lactobacillus salivarius*, K9 – *Lactobacillus rhamnosus*, K10 – *Lactobacillus paracasei*, K11 – *Lactobacillus casei*, K12 – *Lactobacillus reuteri*, K13 – *Micrococcus* spp., K14 – *Streptococcus agalactiae*, K15 – *Enterococcus faecium*, K16 – *Lactococcus lactis*, K17 – *Propionibacterium propionicus*, were pre-selected for this study. They were isolated from the gastrointestinal tract of 500 healthy persons and cancer patients of different age groups (Russian Federation). The study of the gastrointestinal contents of people does not require special permits because the work with isolated microorganisms was performed on human feces; hence, ethical issues are not involved in this case.

Pure cultures were stored at $4\pm 2^{\circ}\text{C}$ lyophilized. Before determination, the strains were pre-cultured twice in anaerobic conditions (fermenter Biostat A plus MO, Sartorius, USA) in MRS-broth for 24 hours at 37°C .

The overnight cultures of microorganisms were harvested by centrifugation (3500 rev/min, 30 min, 4°C) and washed twice with phosphate buffered saline (PBS). The cell concentration was adjusted to 10^5 and 10^7 CFU/ml, respectively; the cells were obtained by heating the bacteria to 95°C for 1 hour. After the heat treatment, the cells were washed with PBS and re-suspended on a suitable medium for cell viability assays. Cell cultures were centrifuged for fractionating (3500 rev/min, 30 min, 4°C).

The cell pellet was washed and re-suspended in 100 ml of PBS for 20 min (at a minute interval); sonication was performed in chilled water (4°C).

Cell debris were removed by centrifugation (14 000 rev/min, 1 hour); supernatants were sterilized by filtration (pore size of 0.22 μm ; Sartorius, Goettingen, Germany). Exponential LAB cultures in MRS broth were used as an inoculum for the anti-cancer tests as follows: cancer cells were grown with cell suspensions containing the harvested

cells of lactic acid bacteria ($\sim 10^5$ or 10^7 CFU/ml of the logarithmic growth phase of the culture of each strain consortium) for 24 hours and then transferred into the chamber.

Culture media:

- MRS medium (bacto-peptone – 10.0 g/l; meat extract – 10.0 g/l; yeast extract – 5.0 g/l; glucose – 20.0 g/l; tween – 1.0 g/l; ammonium citrate – 2.0 g/l; sodium acetate – 5.0 g/l; disodium phosphate – 2.0 g/l; magnesium sulfate 7-water – 0.1 g/l; manganese sulfate 5-water – 0.05 g/l);

- nutrient medium No. 1 (g/l): yeast extract – 5.0; pancreatic casein hydrolyzate – 30.0; papain digest of soy flour – 5.0; sodium hydrogen phosphate – 1.0; twin 80 – 1.0; lactose – 2.5; ammonium citrate – 2.0; cysteine hydrochloride – 1.0; magnesium chloride – 0.5; ascorbic acid – 0.5; sodium acetate – 0.3; sodium chloride – 2.0; glucose – 7.5; meat extract – 10.0; manganese sulfate – 0.05; sodium azide – 0.2; agar – 12.0. Culture conditions: temperature $37.0 \pm 2.0^\circ\text{C}$, pH 7.2 ± 0.2 , duration 12.0 h;

- nutrient medium No. 3 (g/l): yeast extract – 5.0; proteose – 3.0; pancreatic casein hydrolyzate – 25.0; papain digest of soy flour – 7.5; sodium hydrogen phosphate – 2.0; lactose – 2.0; ammonium citrate – 2.0; cysteine hydrochloride – 0.5; magnesium chloride – 0.5; ascorbic acid – 0.2; sodium chloride – 4.0; glucose – 5.0; meat extract – 15.0; manganese sulfate – 0.02; sodium azide – 0.1; agar – 15.0. Culture conditions: temperature $37.0 \pm 2.0^\circ\text{C}$, pH 7.0 ± 0.2 , duration 12.0 h;

- nutrient medium No. 5 (g/l): yeast extract – 5.0; papain digest of soybean meal – 10.0; sodium hydrogen phosphate – 1.0; twin 80 – 0.2; ammonium citrate – 1.0; cysteine hydrochloride – 0.5; magnesium chloride – 0.5; ascorbic acid – 0.5; glucose – 10.0; meat extract – 20.0; manganese sulfate – 0.05; sodium azide – 0.2; agar – 15.0. Culture conditions: temperature $37.0 \pm 2.0^\circ\text{C}$, pH 6.8 ± 0.2 , duration 12.0 h;

- nutrient medium No. 6 (g/l): yeast extract – 10.0; pancreatic casein hydrolyzate – 20.0; sodium hydrogen phosphate – 2.5; twin 80 – 0.7; lactose – 5.0; ammonium citrate – 0.5; cysteine hydrochloride – 1.0; magnesium chloride – 1.0; ascorbic acid – 0.1; sodium acetate – 0.2; sodium chloride – 3.0; glucose – 5.0; meat extract – 15.0; manganese sulfate – 0.1; sodium azide – 0.2; agar – 12.0. Culture conditions: temperature $37.0 \pm 2.0^\circ\text{C}$, pH 7.0 ± 0.2 , duration 12.0 h;

- nutrient medium No. 7 (g/l): proteose – 15.0; papain digest of soybean meal – 10.0; sodium hydrogen phosphate – 1.5; twin 80 – 1.0; ammonium citrate – 2.0; cysteine hydrochloride – 1.0; magnesium chloride – 0.5; ascorbic acid –

0.2; sodium chloride – 2.5; glucose – 10.0; meat extract – 10.0; sodium azide – 0.2; agar – 15.0. Culture conditions: temperature $37.0\pm 2.0^{\circ}\text{C}$, pH 7.0 ± 0.2 , duration 12.0 h.

Biocompatibility of lactobacilli

The biocompatibility was studied by co-cultivation on a solid MRS medium. Overnight culture, grown on a liquid medium and standardized by turbidity standard, was applied on the surface of a solid nutrient medium with a bacteriological loop with diameter of 3 mm.

After the drop soaked, a drop of the other test culture of the same volume was applied on the same medium 1-2 mm away from the edge in the direction of the surface. While spreading, the second drop covered about half of the first drop.

At the overlay, the cultures were developing in mutual presence (co-cultivation), competing with each other. After the second drop dried, the inoculated cups were turned upside down and incubated at $37\text{-}39^{\circ}\text{C}$ in air with an increased concentration of carbon dioxide. After the second drop dried, the cups were turned upside down and incubated at $37\text{-}39^{\circ}\text{C}$ in air with an increased concentration of carbon dioxide. Each experiment was repeated with a changing position of inoculations (to avoid the impact of successive layering of drops on the growth pattern in the area of co-cultivation).

Drops of the same culture, layered on top of each other as described above, were used as control. The results were interpreted 24 and 48 hours after the start of incubation.

In case of growth inhibition of one of the cultures, the relations between these cultures were regarded as antagonistic and the cultures were referred to the category of bioincompatible. Cultures were considered biocompatible in case of a full “merging” of spots or enhanced growth of the strains in the area of co-cultivation (mutualism, synergy, satellism).

If one of the cultures in the area of co-cultivation “rises” and inhibits the growth of the second culture, regardless of the order in which they were applied, then such an option was considered weak antagonism. The presence of a well-defined zone of inhibition (growth retardation) of one culture by another test culture on the spot periphery was regarded as a sign of strong antagonism.

Minimum inhibitory concentration

The minimum inhibitory concentration was determined against bacterial strains isolated with the microtiter method [21].

Cell culture and adhesion to cancer cells

The following cancer cells were used as test-cultures: Burkitt lymphoma LBR2, human prostate cancer DU 145 human breast cancer MDAMB-231 and MCF7, hepatocellular carcinoma HepG2, brain cancer U-87, and human pancreatic cancer PANC-1 from the collection of the Cancer Center Karolinska (Sweden).

The cells were cultured at 37°C and 5% CO₂ on a RPMI 1640 medium (PanEco, RF) containing 10% fetal bovine serum (HyClone Laboratories, Logan, UK), inactivated at 56°C for 30 minutes, 2 mM of L-glutamine, 100 µg/ml of penicillin and 100 µg/ml of streptomycin sulfate (PanEco, RF). Light microscopy of cells was performed using AxioVision 4 system (Zeiss, Germany). Cell viability was determined by trypan blue dye exclusion (PanEco, RF) in the Gorjaev's chamber.

The tumor cells that reached the logarithmic growth phase were transferred into 96-well flat-bottomed microplates («Costar») 5•10⁴ - 6,5•10⁴ cells per well, and pre-incubated for 24 h before adding test organisms under the conditions of 5% CO₂ and 37°C.

The obtained cell cultures in a wide range of progressively decreasing concentrations were added to wells with the cell culture (20 µl to 180 µl of cell suspension) and co-incubated for 48 hours. 0.9% sodium chloride solution in an adequate amount (20 µl) was added to the control wells. The number of living cells in the wells at the end of the incubation period was determined by the MTT colorimetric method.

Antiproliferation of colon cancer cells

MTT assay

The MTT colorimetric method is based on the ability of the dehydrogenase of living cells to restore 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) to purple formazan crystals, soluble in dimethylsulfoxide. Optical absorption of dyed solutions of dimethylsulfoxide was measured with the Multiskan MS tablet photometer (Labsystem, Finland) at λ=540 nm. Cytotoxicity of the test cell cultures was evaluated according to the following formula:

$$[\% \text{ of cytotoxicity} = (1 - \text{optical absorption in the test sample} / \text{optical absorption in the control}) * 100].$$

Trypan Blue exclusion assay

Cancer cells (10⁶ cells/1 ml of cell suspension) were added to a 96-well plate. The suspension was incubated at 37°C for 24 hours. Cell viability was examined using Trypan Blue exclusion:

$$[\% \text{ cell viability} = (\text{live cell count} / \text{total cell count}) * 100].$$

Statistical analysis

All repeating experiments were performed three times. Data treatment was carried out using standard methods of mathematical statistics. Differences between means were considered significant when the confidence interval was smaller than 5% ($P \leq 0.05$).

Results

Composition lactic acid bacteria consortia

Seventeen strains of lactic acid bacteria, indicated as K1, K2, K17, were isolated from the gastrointestinal tract of 500 healthy persons and cancer patients of different age groups (Russian Federation). In order to extend the range of effects, the biocompatibility of the isolated lactic acid bacteria was studied and symbiotic consortia, the metabolites of which have antitumor properties, were composed.

The biocompatibility of the selected strains among themselves in combinations: 1) K1, K2, K5, K6, K7, K8, K13, K14; 2) K3, K4, K15, K9, K10, K11, K12, K16, K17, - was studied using the method of co-culturing on solid nutrient media, typical for studied strains. The results are presented in the **Table 1**.

Table 1: Biocompatibility of microorganism strains isolated from the human intestinal tract.

Name of the strain	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	
K1	-	BC	BC	WA	BC	BC	BC	WA	BC	BC	BC	BC	BC	BI	WA	BI	WA	WA
K2	BC	-	BC	BC	BC	BC	BC	BC	WA	BC	BC	WA	BC	BC	WA	WA	WA	WA
K3	BC	BC	-	BC	BI	WA	BI	BC	BC	BC	BC	WA	BC	BC	BI	BC	WA	WA
K4	WA	BC	BC	-	WA	WA	BI	WA	BC	BC	BC	BC	WA	BC	WA	WA	WA	WA
K5	BC	BC	BI	WA	-	BC	WA	BC	BC	BC	BC	BC	BC	BC	BC	BI	BC	WA
K6	BC	BC	WA	WA	BC	-	BC	BC	BC	BC	BC	BC	BC	WA	WA	BC	WA	WA
K7	BC	BC	BI	BI	WA	BC	-	BC	BC	BC	BC	WA	BC	BC	WA	WA	BI	BI
K8	WA	BC	BC	WA	BC	BC	BC	-	BC	BI	WA	BC	BI	BC	BC	WA	BC	BC

K9	BC	WA	BC	BC	BC	BC	BC	BC	-	BC	BC	BC	WA	WA	BC	WA	BI	
K10	BC	BC	BC	BC	BC	BC	BC	BI	BC	-	BC	WA	WA	BC	BC	BC	BC	
K11	BC	BC	BC	BC	BC	BC	BC	WA	BC	BC	-	BC	WA	WA	BC	BC	BC	
K12	BC	WA	WA	BC	BC	BC	WA	BC	BC	WA	BC	-	BC	BC	BC	BC	BC	
K13	BI	BC	BC	WA	BC	BC	BC	BI	WA	WA	WA	BC	-	BC	BC	BC	BI	
K14	WA	BC	BC	BC	BC	WA	BC	BC	WA	BC	WA	BC	BC	-	BC	BI	WA	
K15	BI	WA	BI	WA	BI	WA	WA	BC	BC	BC	BC	BC	BC	BC	-	WA	BC	
K16	WA	WA	BC	WA	BC	BC	WA	WA	WA	BC	BC	BC	BC	BI	WA	-	BC	
K17	WA	WA	WA	WA	WA	WA	BI	BC	BI	BC	BC	BC	BC	BI	WA	BC	BC	-

* BC - Biocompatible, BI - Bioincompatible, WA - Weak antagonism

It was found that the strains K2 and K11 are biocompatible with all strains of their group, while K5, K6, K7 and K10 can be identified as conditionally biocompatible for the first and second groups, respectively. Bioincompatibility was found for combinations: K1 +K13; K8 + K13.

The consortia from the studied strain interaction microorganisms were composed based on the conducted research. The consortia were indicated as follows: No. 1 - K1, K2, K6, K7, K9, K10; No. 2 - K2, K5, K6, K7, K8, K14, K10, K11; No. 3 - K5, K6, K7, K8, K14, K9, K10; No. 4 - K3, K1, K8, K14, K4, K10; No. 5 - K12, K1, K5, K6, K8, K14, K15. The culturing conditions for the maximum cell viability were as follows: consortium No. 1 - 37.0±2.0°C, pH=6.8±0.2, culture medium No. 5; consortium No. 2 - 37.0±2.0°C, pH=7.0±0.2, culture medium No. 7; consortium No. 3 - 37.0±2.0°C, pH=7.0±0.2, culture medium No. 3; consortium No. 4 - 37.0±2.0°C, pH=7.0±0.2, culture medium No. 1; consortium No. 5 - 37.0±2.0°C, pH=7.0±0.2, culture medium No. 6.

The antitumor properties of microbial consortia were tested *in vitro* on cancer cell lines (Cancer Center Karolinska, Sweden): Burkitt's lymphoma LBR2, human prostate cancer DU145, human breast cancer MDAMB-231 and MCF7, hepatocellular carcinoma HepG2, brain cancer U-87, and human pancreatic cancer PANC-1. The results of the study

of antitumor activity of the studied consortia are presented in **Figure 1**. All studied microbial consortia are characterized by antitumor activity against the tested cell lines; however, but consortia No.1 and No. 3 have the strongest cytotoxic properties.

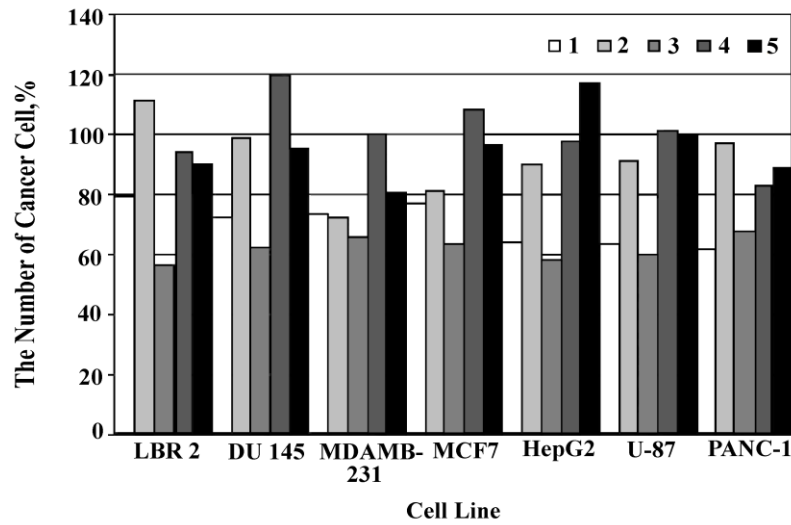


Figure 1 A. The survival rate of cancer cells treated with a concentration of cell cultures 10^5 CFU/ml of lactobacilli consortium: 1 – consortium No. 1; 2 – consortium No. 2; 3 – consortium No. 3; 4 – consortium No. 4; 5 – consortium No. 5.

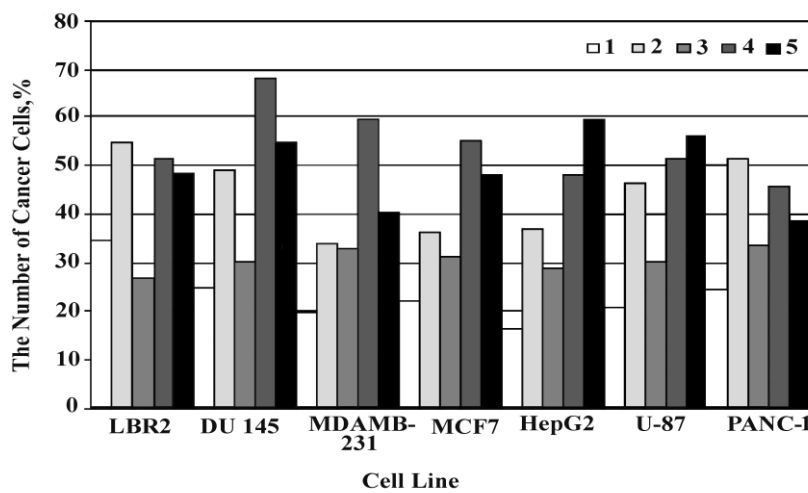


Figure 1B. The survival rate of cancer cells treated with a concentration of cell cultures 10^7 CFU/ml of lactobacilli consortium: 1 – consortium No. 1; 2 – consortium No. 2; 3 – consortium No. 3; 4 – consortium No. 4; 5 – consortium No. 5.

Consortium No. 1 at a concentration of 10^7 CFU/ml shows the strongest activity against the cell lines of hepatocellular carcinoma HepG2 (16.4% survival) and human breast cancer MDAMB-231 (19.6% survival). Consortium No. 3 at a concentration of 10^7 CFU/ml shows the strongest activity against the cell lines of Burkitt's lymphoma LBR2 (26.8% survival) and hepatocellular carcinoma HepG2 (28.9% survival).

Discussion

Probiotics have a positive effect on the immune system, since gastrointestinal flora reduces the impact of mutagens and carcinogens. The present study investigated the biocompatibility of 17 strains of lactic acid bacteria previously isolated from the gastrointestinal tract of healthy people and cancer patients in combination with each other: 1) *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Micrococcus* spp., *Streptococcus agalactiae*; 2) *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Enterococcus faecium*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactococcus lactis*, *Propionibacterium propionicus*. It was found that the biocompatibility of all strains of the group is typical for *Bifidobacterium breve* and *Lactobacillus casei* strains, conditional biocompatibility can be distinguished for *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus paracasei* for the first and second groups, respectively. Bioincompatibility was found for combinations: *Bifidobacterium bifidum* + *Micrococcus* spp.; *Lactobacillus salivarius* + *Micrococcus* spp.

The study of antitumor properties of lactic acid bacteria consortia composed of 17 previously selected probiotic strains demonstrated strong antitumor activity of the consortia to cancer cell lines, Burkitt's lymphoma LBR2, human prostate cancer DU145, human breast cancer MDAMB-231 and MCF7, hepatocellular carcinoma HepG2, brain cancer U-87, and human pancreatic cancer PANC-1. It was found that the strongest cytotoxic properties were typical for consortium No.1 (*Bifidobacterium bifidum*, *Bifidobacterium breve*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*) and No. 3 (*Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Streptococcus agalactiae*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*). The discovered regularities enable using the studied consortia to create functional foods for the rehabilitation of cancer patients. Further research is needed to determine the nature and mechanisms of inhibition of lactic acid bacteria, including on cancer cell cultures. Known probiotic and antitumor characteristics of lactic acid bacteria, including those isolated from the human gastrointestinal tract, are possible conditions for using them as a basis for the functional food for cancer patients during rehabilitation.

The herein obtained results on biocompatibility and antitumor activity of lactic acid bacteria isolated from the human gastrointestinal tract should be used when engineering functional food for cancer patients during rehabilitation, since probiotics are an integral part of the diet of cancer patients. Cytostatic and radiation therapy used in patients with

cancer, having a small threshold between therapeutic and toxic doses, causes damage to the gastrointestinal tract, its mucous membranes, which are extremely susceptible to the damaging effects because they are actively proliferating tissues, and to the microbial intestinal flora, which is a rapidly proliferating community. Normal intestinal flora is destroyed; the range of potentially pathogenic microorganisms expands; the number of members and species changes. All this aggravates the damage to the gastrointestinal tract and liver, which determines the degree of somatic decompensation and requires timely diagnosis, dictates the need to search and include in the complex of rehabilitation measures preparations aimed at restoring the normal gut microbiota, which can be achieved by including products rich in probiotics in the diet.

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