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OPTIMIZATION OF EXPERIMENTAL MODEL SYSTEMS FOR EVALUATING RECIPROCAL INFLUENCE OF *BIFIDOBACTERIUM ANIMALIS* AND HUMAN BREAST CANCER CELLS *IN VITRO*

Background. The development of human breast cancer (BC) is known to be closely related to disturbances in the mammary gland microbiota. Bacteria of the genus *Bifidobacterium* are an important component of normal breast microbiota and exert antitumor activity. The molecular-biological mechanisms of interaction between BC cells and microbiota members remain poorly studied yet. **The aim** of this study was to develop and optimize an experimental model system for the co-cultivation of BC cells with *Bifidobacterium animalis in vitro*. **Materials and Methods.** Human BC cells of the MCF-7, T47D, and MDA-MB-231 lines, as well as live and heat-inactivated bacteria of *Bifidobacterium animalis subsp. lactis (B. animalis)* were used as research objects. The growth kinetics and viability of *B. animalis* in the presence of different BC cell lines and without them were determined by both the turbidimetry method and seeding on an elective nutrient medium. Glucose consumption and lactate production by bifidobacteria were assessed by biochemical methods. The viability of BC cells was determined by a standard colorimetric method. **Results.** The growth kinetics of *B. animalis* in the complete DMEM nutrient medium showed standard patterns. The indicators of glucose consumption and lactate production of *B. animalis* confirm its physiological metabolic activity under the growth conditions. The presence of BC cells in the model system did not affect the duration of the growth phases of the *B. animalis* cells' population but contributed to the increase in their counts. A significant decrease in the number of live BC cells of all studied lines was observed only after 48 h of co-cultivation with live *B. animalis.* To achieve similar suppression of the BC cell viability, 10—30-fold higher counts of heat-inactivated bacteria were required compared to live ones. **Conclusions.** The optimal conditions for co-cultivation of human BC cells and living *B. animalis* cells *in vitro* have been identified.

Keywords: breast cancer, *Bifidobacterium*, microbiota, growth curve, pH, cell culture.

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The current metabolomic and metagenomic studies evidence that the development of breast cancer (BC) is closely related to the disturbances in the microbiota of the breast tissue and intestines [1]. The breast tissue and ducts are colonized by a diverse community of microorganisms originating from the skin of the breast, nipples, areolae, as well as enteric microbiota. The mammary gland has own microbiota, the composition of which differs from the microbiota of other biotopes of the human body. At the same time, lactic acid anaerobic bacteria of the genus *Bifidobacterium* are an important component of the breast microbiota, although they are members of the normal gut microbiota. However, presently it is accepted that non-pathogenic intestinal bacteria can be transported to other biotopes. In particular, dendritic cells can transport intestinal microbiota to human breast tissue [2]. Also, the translocation of bifidobacteria from the intestine to the mammary gland can occur due to the increased permeability of the intestinal epithelium caused by the physiological and hormonal changes during pregnancy [3, 4].

As known, the antitumor effects of probiotic strains of bifidobacteria, in particular *Bifidobacterium animalis*, are mediated by their influence on the host's immunity and/or by the direct effect on tumor cells. In particular, through the production of metabolites such as short-chain fatty acids, which have powerful immunomodulatory properties and can inhibit the proliferation of malignant cells [5], induce cytokine production and apoptosis [6, 7], and inhibit the growth of experimental tumors *in vivo* [8].

Despite significant progress in studying the specifics of the interaction between malignant cells and the members of the human microbiota at the cellular level, the molecular biological mechanisms of such interaction have not been definitively elucidated and require further research. To study such mechanisms, *in vitro* models of the cancer cell cultivation with bacteria are an extremely promising option, as they allow one to clarify various aspects of their mutual influence on the biological properties of both types of cells in a controlled environment [9] and to separate direct and indirect effects of microbiota on eukaryotic cells.

Quite often, heat-inactivated microorganisms are used as elements of such experimental *in vitro* models, since their number does not change during the entire experiment and is easily controlled. It is also known that heat-inactivated bacterial cells preserve the integrity of the cell wall components, which allows them to partially preserve their antigenic properties when interacting with eukaryotic cells including the immune system and tumor cells [10]. The disadvantage of such a model system is the impossibility of using it to study the biological effects of bacterial metabolites.

Therefore, the informativeness and the value of the experiments on *in vitro* models with living microorganisms are much higher due to their potent biological effects [11—14]. The interaction of eukaryotic cells with heat-inactivated bacteria is limited to physical contact, the consequence of which is the antigenic effects of microorganisms. In the case of living bacteria, the interaction is realized due to the physical contact between cells, as well as distantly due to the metabolic effects [15].

However, experimental models of co-cultivation of various types of living cells *in vitro*, in particular, cultures of human BC cells and bifidobacteria, are extremely complex multicomponent systems that require a robust experiment design [16]. The situation is complicated by the fact that the standard nutrient cultivation medium for eukaryotic cells and their growth conditions are not convenient for the culturing of bifidobacteria, which require specific growth factors [17].

Hence, the aim of our work was to develop and optimize an experimental model system for the co-culturing of human BC cells and *B. animalis*.

Materials and Methods

Bacterial strain. We used the lyophilized *Bifidobacterium animalis subsp. lactis* BB-12 strain diluted in physiological solution to a final density of 3×10^8 cells/ml. Bacterial cells were isolated from the probiotic manufactured by Lek Pharmaceuticals, Ljubljana, Slovenia.

Cell lines. As a model of BC, we chose three human BC cell lines with different morphological and molecular biological characteristics described in [18]. All cell lines were provided by the Bank of Cell Lines from Human and Animal Tissues of the R. E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology of the NAS of Ukraine. The cells were maintained in complete DMEM medium with 4 mmol/l L-glutamine (BioWest, France), 10% fetal bovine serum (FBS) (BioWest, France) without antibiotic at 37 °C in a humidified atmosphere with 5% CO_2.

Co-culturing of bacteria with human BC cells. The number of *B. animalis* cells in the presence of human BC cells and without them was determined by the kinetics of the cell growth through measuring the optical density (OD_{600}) as well as by seeding on elective nutrient medium at certain time points. For this, human BC cells were plated into the wells of 96-well plates (SPL, Korea) in the complete DMEM medium at a density of 0.7×10^4 cells/well and cultured for 10 h. Then the medium was replaced with a fresh one, and bifidobacteria were introduced at the ratio (BC cells to *B. animalis* cells ratio, BC/BAC) of 1/400, 1/100, and 1/40, and cultivation continued under the same conditions. The initial BC/BAC ratio was used for marking the corresponding samples throughout the study, regardless of its change in the growth kinetics. A DMEM medium without eukaryotic cells served as a control of the bifidobacteria growth.

The kinetics of the bacterial growth was evaluated by turbidimetry method for 72 h (every 2 h from 0 h to 28 h and every 3—5 h from 30 h to 72 h) on a Labsystems Multiskan PLUS spectrophotometer (Finland) at $\lambda = 600$ nm, according to the method [19].

The number of viable cells of *B. animalis* was determined by seeding their 10-fold dilutions in a physiological solution in test tubes with selective medium for bifidobacteria (Bifidum medium, Farmaktiv, Ukraine) at 0, 6, 12, 24, 48, and 72 h after the introduction of bacterial cells into the wells. The cultures were incubated for 72 h at 37 °C, then the number of colonies was counted and recalculated per 1 ml of the original medium. The results were presented as the number of colony forming units per ml (CFU/ml).

Glucose consumption and lactate production by *B. animalis* were determined in the DMEM medium accounting the initial and final concentrations of glucose and lactate. The glucose content was determined by the colorimetric enzymatic method with glucose oxidase, using the "Diagnostic kit for the determination of glucose concentration" (CORMAY, Poland), according to the manufacturer's instructions. The lactate content was determined by the enzymatic method with lactate oxidase using the "Diagnostic kit for determining the concentration of lactate" (CORMAY, Poland) according to the manufacturer's instructions. An automatic biochemical analyzer GBG ChemWell 2900 (Awareness Technology, USA) was used to evaluate the results.

Analysis of pH. pH of the culture medium was determined at time points 0, 6, 12, 24, 48, and 72 h after the introduction of *B. animalis* in the wells using a pH meter PHS-3C (Kelilong Electron, China) according to the manufacturer's instructions.

Inactivation of bacteria. Lyophilized bacteria *B. animalis* were added to the DMEM medium to a final density of 1×10^6 CFU/ml and incubated at 37 °C in a humidified atmosphere with 5% CO_2. After 24 h, the bacteria were washed twice with a phosphate-buffered saline (BioWest, France) and diluted in a physiological solution (Lekhim, Ukraine). A sample of the bacterial suspension was taken to determine the number of bifidobacteria by the microbiological method,

as described above. The rest of the bacterial cells were subjected to heat inactivation by heating in a water bath at 72 °C for 30 min [20]. The absence of viable bacteria after inactivation was confirmed by seeding of a sample of inactivated bacteria in elective Bifidum medium.

Cell viability. To assess the effect of live *B. animalis* on the survival of human BC cells, the cells were plated in the wells of a 96-well plate, and live bacterial cells were introduced as indicated above. After 6, 12, 24, 48, and 72 h of co-cultivation, the viability of cancer cells was determined by the colorimetric method and staining with crystal violet (Sigma, USA).

To assess the effect of heat-inactivated bifidobacteria on the viability of BC cells, the cells were plated in the wells of a 96-well plate with the complete DMEM medium at a density of 0.7×10^4 cells/well and incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 10 h. Then the medium was replaced by a fresh one, and heat-killed bifidobacteria were introduced at BC/BAC ratios of 1/300—1/12000. The cells were incubated for another 48 h at the same conditions. The absorbance was measured using the Labsystems Multiskan PLUS spectrophotometer at 540 nm. All experiments were performed in triplicate and the results were expressed as a number of viable cells in the experimental group relative to control (%) [21]:

$$X = \frac{\text{A540 } (experiment)}{\text{A540 } (control)} \times 100\%$$

Statistical analysis. The means (M) and standard deviations (SD) were calculated. The significance of the differences in means was analyzed by an unpaired *t*-test with Welch`s correction. The growth kinetics of *B. animalis* was analyzed using the Weibull mathematical model:

$$N_t = N_{max} - (N_{max} - N_0) * \exp(-(k * t)d)$$

where N_t is the number of *B. animalis* cells at time t; N_{max} is the maximum number of *B. animalis* cells during observation; N_0 is the number of *B. animalis* cells at the initial time point; t is the observation time (hours); k characterizes the rate of cell division in the exponential phase; d characterizes the effect of spatial heterogeneity in cell growth on the rate of division in the exponential phase. The model parameters were determined by a nonlinear regression from the best approximation of the model to the experimental data. The differences were considered significant at $p < 0.05$.

Results

Growth kinetics of B. animalis in complete DMEM medium. The growth kinetics of *B. animalis* in DMEM medium with different initial bacterial counts in inoculums, which further on corresponded to the BC/BAC of 1/400, 1/100, and 1/40, followed the classical mode (Fig. 1). The duration of the lag phase depended inversely on the initial inoculum counts taking from 2 h to 4—6 h and was less dependent on the initial counts of bifidobacteria.

So, after 24 h of incubation, bacteria of all studied variants entered the stationary phase of growth. The final count of bifidobacteria also depended on the count in the initial inoculums. The maximum count of live bacterial cells observed after 24 h of inoculation of *B. animalis* was $(100.0) \times 10^5$ CFU/ml (1/400), $(55.0 \pm 2.7) \times 10^5$ CFU/ml (1/100), and $(7.8 \pm 0.6) \times 10^5$ CFU/ml (1/40). After 72 h of incubation, the viability of the bacterial cells decreased by 10%—15%.

Metabolic activity of B. animalis. The metabolic activity of *B. animalis* in DMEM medium was determined by measuring the glucose consumption and the production of lactate. As shown in Fig. 2, bifidobacteria most intensively consumed glucose during the first 6—24 h of cultivation in DMEM, and at later terms, the intensity of glucose metabolism did not change significantly. The lactate production by *B. animalis* cultured in DMEM increased significantly

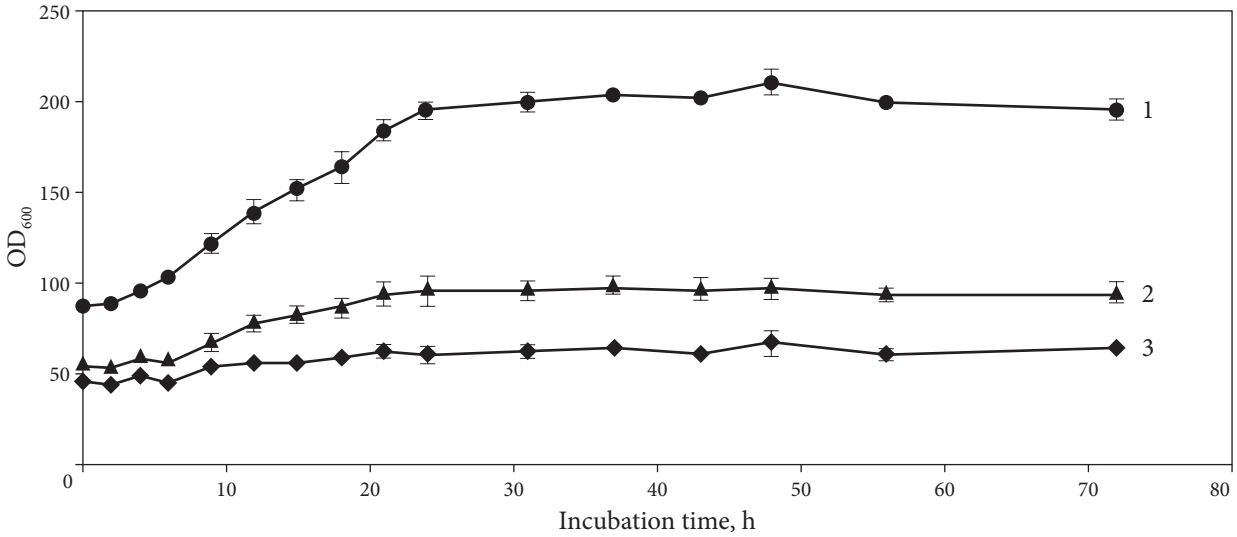

Fig. 1. Growth kinetics of *B. animalis* cultured in complete DMEM medium for 72 h at different initial inoculum counts: 1 — (28.0 ± 1.7) × 10^5 CFU/ml (2 — (6.5 ± 0.9) × 10^5 CFU/ml; 3 — (2.5 ± 0.3) × 10^5 CFU/ml. The data presented in the graph (OD600) were obtained by a turbidimetry method. At the same time, the number of viable bacterial cells (CFU/ml) in the wells at the initial time point and in the course of bacterial growth was also determined by a microbiological method

for 6—48 h of cultivation. Fig. 2 shows the metabolic activity of bacteria inoculated at the largest count (28.0 ± 1.7) × 10^5 CFU/ml (corresponds to 1/400 BC/BAC). The patterns of the metabolic activity of bacteria inoculated at other initial counts were similar.

pH of the culture medium in the growth kinetics of *B. animalis* inoculated at (28.0 ± ± 1.7) × 10^5 CFU/ml (1/400) decreased significantly ($p < 0.05$) from 7.60 to 7.36 and 7.33 after 48 h and 72 h of cultivation, respectively. Bacteria grown at other inoculation doses did not affect pH of the culture medium.

Growth of B. animalis co-cultivated with human BC cells. When comparing the growth kinetics of *B. animalis* under the conditions of co-cultivation with different BC cell lines, we found that at the 1/400 and 1/100 BC/BAC ratios, there was a tendency to increase in bacterial counts in comparison with bifidobacteria grown in DMEM only (Fig. 3). Such changes were especially pronounced in the case of co-cultivation with MDA-MB-231 cells at an initial BC/BAC ratio of 1/400 (Fig. 3, *a*).

A statistical analysis of the comparative assessment of the *B. animalis* growth kinetics with

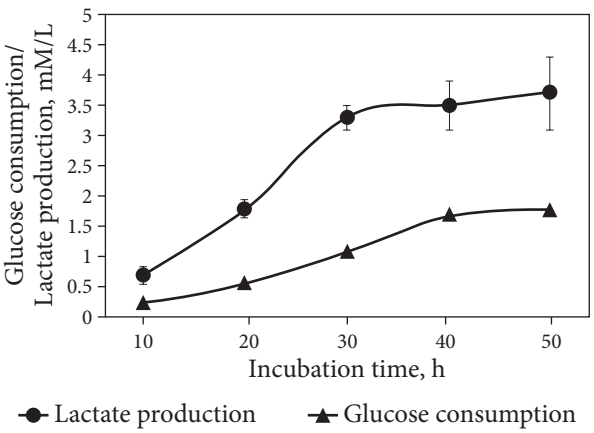

Fig. 2. Glucose consumption and lactate production by *B. animalis* in DMEM medium

or without BC cells was carried out using the Weibull mathematical model. Table 1 presents the results of such an analysis. The parameter A increased after co-cultivation of bacterial and cancer cells (BC/BAC ratio 1/400) mainly due to the acceleration of the division of *B. animalis* in the exponential growth phase.

After exposure of BC cells with *B. animalis* at the 1/100 ratio, statistically significant differences in the number of *B. animalis* cells in the sta-

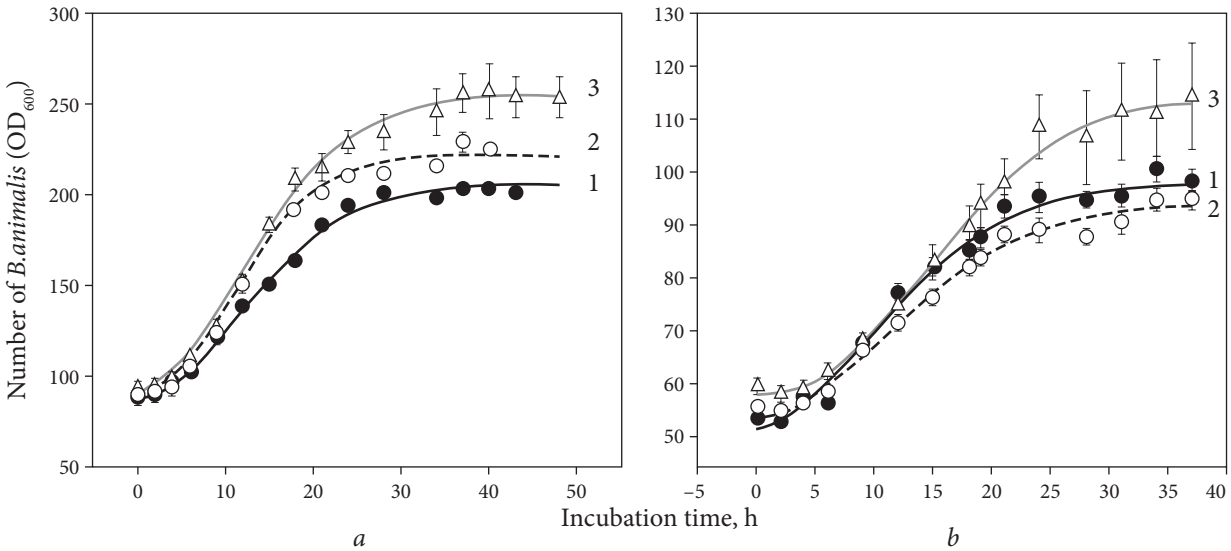

Fig. 3. Growth kinetics of *B. animalis* co-cultured with human BC cells at the initial BC/BAC ratio of 1/400 (a) and 1/100 (b): 1 — *B. animalis* only; 2 — co-cultivation with MCF-7/T47D; 3 — co-cultivation with MDA-MB-231. The symbols represent the experimental data. The lines are the best approximation of the Weibull mathematical model to the experimental data

tionary phase of growth were noted when they were co-cultivated with MDA-MB-231 cells.

A significant decrease in the number of living BC cells of all studied lines in comparison with the control was observed only after 48 h of co-culturing with bacteria at the initial BC/BAC ratios of 1/400 and 1/100 ($p < 0.05$). The MCF-7 and T47D cell lines were the most sensitive to bifidobacteria: a decrease in the number of live MCF-7 cells by 25%—65% and T47D cells by 20%—35% was observed compared to the control (Fig. 4). After 72 h of co-cultivation of BC cells with bifidobacteria at an initial ratio of 1/400, the death of 95% of all BC cells was observed (data not presented).

To assess the possible changes in the BC/BAC ratio after 48 h of co-cultivation, we analyzed the number of bacterial cells by spectrophotometry and by the method of serial dilutions (Table 2). The different speed and density of growth of eukaryotic and bacterial cells led to the noticeable changes in the BC/BAC ratio compared to the initial ones. This is an important index because it can help explain the described biological effects.

Table 1. **Parameters of the mathematical model of the *B. animalis* kinetic growth during co-cultivation with BC cells at a BC/BAC ratio of 1/400**

Model parameters	*B. animalis*	*B. animalis* + MDA-MB-231	*B. animalis* + MCF-7	*B. animalis* + T47D
A (OD_{600})	205.2 ± 1.9	254.6 ± 2.5*	222.0 ± 2.9*,#	219.7 ± 1.6*,#
B (OD_{600})	87.4 ± 3.5	88.8 ± 4.5	84.9 ± 4.9	88.5 ± 3.0
d	1.93 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	2.3 ± 0.2
k (h-1)	0.06 ± 0.002	0.06 ± 0.002	0.07 ± 0.003*	0.075 ± 0.002*

Notes: A (OD_{600}) is the optical density reflects the number of *B. animalis* cells in the stationary phase of growth; B (OD_{600}) is the optical density reflects the number of *B. animalis* cells at the initial point (0 h); * $p < 0.05$ as compared to the counts of *B. animalis* only; # $p < 0.05$ as compared to the counts of *B. animalis* + MDA-MB-231 cells.

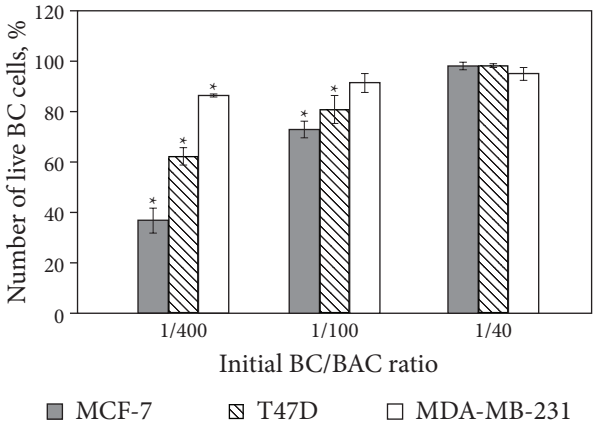

Fig. 4. Viability of BC cells after exposure to *B. animalis* for 48 h. * $p < 0.05$ as compared to the untreated cells

pH changes during co-cultivation of *B. animalis* with human BC cells were analyzed for 72 h. Already after 12 h of incubation, a significant decrease in pH ($p < 0.05$) was observed in co-cultivation of MCF-7 and MDA-MB-231 cells with bifidobacteria at a ratio of 1/400 (15% and 35% decrease in pH for both BC cell lines after 48 and 72 h, respectively). pH values were 7.2—7.3 after 24 h of co-cultivation, 6.4—6.8 after 48 h, and 4.5—5.7 after 72 h.

Viability of human BC cells exposed to heat-inactivated B. animalis. Exposure of MCF-7 cells to heat-inactivated *B. animalis* led to a significant decrease in the number of live cells by 25%—75% at BC/BAC ratios of 1/3000—1/12000 ($p < 0.05$). Treatment of T47D cells with bifidobacteria was accompanied by a significant decrease in the number of live BC cells by 10%—20% at BC/BAC ratios of 1/1200—1/12000 ($p < 0.05$). MDA-MB-231 cells were the least sensitive to *B. animalis*: a significant decrease in the cell viability was noted only after their exposure to bacteria at a BC/BAC ratio of 1/12000 ($p < 0.05$).

Table 2. **Changes in the BC/BAC ratio after 48 h of co-cultivation**

BC cells	BC/BAC ratio	
	0 h	48 h
MCF-7	1/400	1/935
	1/100	1/136
	1/40	1/3
T47D	1/400	1/735
	1/100	1/200
	1/40	1/6
MDA-MB-231	1/400	1/200
	1/100	1/70
	1/40	1/2

Discussion

The studies of the components of the microenvironment of malignant neoplasms of the human mammary gland testify to the important role of the microbiota in the formation of the unique tumor phenotype [22, 23]. Lactic acid bacteria of the genus *Bifidobacterium* with their antitumor, anti-inflammatory, and immunomodulatory activities are among the most studied microbiota members of both the human intestine and mammary gland [24, 25].

To study the features and consequences of the interaction between microbiota members, in particular bifidobacteria, and malignant cells, various experimental *in vitro* models are widely and successfully used today [26, 27]. In such studies, inactivated forms of bacterial cells are often used. The only significant advantage of such models is a constant number of bacteria during the entire time of cell incubation. However, under such experimental conditions, it is impossible to study the effect of secreted metabolites of microbiota members on eukaryotic cells, which is an extremely important aspect of their interaction in vivo. After all, it has been proven that the human microbiome is in close interaction with the metabolic processes of the body, and the microbiota members and their metabolites exert many independent and diverse effects on the tumor and the body as a whole [22].

That is why, we gave preference to the study of the influence of live bacteria on breast cancer cells. However, under such cultivation condi-

tions, the question of validation of the experimental model arises. It is also important to take into account that bifidobacteria are anaerobic sucrolytic lactic acid bacteria dependent on the physical and chemical conditions of the environment and the composition of the nutrient medium [28].

The growth kinetics of *B. animalis* in the complete DMEM medium showed standard patterns possibly due to the presence of free amino acids and vitamins, which bifidobacteria use during the first 24 h of incubation [29]. At the later stages of growth, bifidobacteria can use peptides as a source of nutrients only if they possess a certain proteolytic activity [30]. According to the literature, some strains of bifidobacteria, in particular *Bifidobacterium animalis subsp. lactis*, do not possess proteolytic activity [31]. Therefore, they are not able to independently metabolize peptides, which can explain only one and short-term log-phase of bacterial growth under the specified conditions.

Another important indicator of the viability of *B. animalis* cells, which describes their growth characteristics, is their metabolic activity, in particular, glucose consumption and lactate production. It is known that in bifidobacteria, monosaccharides are metabolized by the so-called fructose-6-phosphate or bifid shunt [32]. Fructose-6-phosphate phosphoketolase is a characteristic enzyme of this pathway, one of the final products of which is lactate [33]. At the same time, if the nutrient medium contains a significant amount of glucose, the main product of the carbohydrate metabolism will be lactate. If the survival of the bacterial cell is threatened due to the deficiency of monosaccharides, the level of lactate production will be low [34]. The data on glucose consumption and lactate production confirmed the physiological metabolic activity of *B. animalis* under the growth conditions in our study. It is important to note that *B. animalis* after 48—72 h of incubation at the highest ratios used in our study metabolize only 16%—17% of the total amount of glucose in DMEM. This proved that the number of bacteria used in the experiment did not cause glucose depletion in the culture medium.

The absence of changes in pH of the culture medium during the growth of bifidobacteria was probably due to the buffer capacity of the CO_2/HCO_3 system, which is provided by the chemical composition of DMEM and the incubation conditions of the cell culture (5% CO_2). In addition, 10% FBS included in the complete nutrient medium can also increase its buffer capacity [35]. Therefore, according to our data, the most optimal conditions for evaluating the effect of live *B. animalis* cells on human BC cells *in vitro* are an incubation time of 48 h and a BC/BAC ratio of > 1/40 and ≤ 1/400.

Our data indicate that the presence of BC cells does not affect the duration of the growth phases of the bacteria, but contributes to an increase in their counts, which can be associated with both the change in pH and the component composition of the nutrient medium due to the vital activity of BC cells [36]. It is known that most probiotic strains of bifidobacteria are characterized by relationships between the growth rate, metabolic activity, and acid formation[37], with a narrow pH optimum (from 6.5 to 7) required for growth [38]. Besides, tumor cells often use a glycolytic pathway of glucose metabolism and, consequently, produce lactate actively [39]. The lactate, in its turn, also affects pH of the medium creating more favorable conditions for the growth of bifidobacteria. This suggestion is confirmed by our previous results. Namely, MDA-MB-231 cells produce the largest amount of lactate to the medium (unpublished data), which results in the most significant increase in the growth activity of *B. animalis* in the presence of BC cells of this line.

Analyzing the effect of *B. animalis* on the number of living BC cells, one should also take into account possible changes in the ratio of eukaryotic/bacterial cells after 48 h of co-cultiva-

tion. The obtained results can explain the higher sensitivity of MCF-7 and T47D cells to the influence of *B. animalis*, since in the experiment with these cells, the BC/BAC ratios shifted toward nearly 2-fold increase in the bacterial cell counts in contrast to MDA-MB-231 cells, characterized by high proliferative activity and the largest growth density.

In the case of co-cultivation of BC cells with living bacteria, eukaryotic cells are affected by both the bacterial metabolites and the direct contact. We have studied the effect of heat-inactivated *B. animalis* on the BC cell viability and revealed that MCF-7 cells were the most sensitive to the influence of inactivated bifidobacteria, and MDA-MB-231 cells were the least sensitive. At the same time, to achieve a similar effect for inhibiting the viability of tumor cells, a 10-30-fold higher counts of heat-inactivated bacteria were required in comparison with live ones. This suggests that an important component of the negative effect of bifidobacteria on the viability of the studied BC cell lines is their antigenic activity, which depends on the expression of the appropriate pattern recognition receptors on tumor cells [40].

Thus, we have evaluated a complex of biological properties of *B. animalis* cells regarding their survival, growth characteristics, and certain indicators of metabolic activity under conditions of co-cultivation with human BC cells, as well as optimal conditions for such co-cultivation. The obtained results can be useful in the study of microbiota as a component of tumor microenvironment and as an important component of supportive therapy in the treatment of cancer patients.

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ОПТИМІЗАЦІЯ ЕКСПЕРИМЕНТАЛЬНИХ МОДЕЛЬНИХ СИСТЕМ ДЛЯ ОЦІНКИ ВЗАЄМОВПЛИВУ *BIFIDOBACTERIUM ANIMALIS* ТА КЛІТИН РАКУ МОЛОЧНОЇ ЗАЛОЗИ ЛЮДИНИ *IN VITRO*

Стан питання. Відомо, що розвиток раку молочної залози (РМЗ) людини тісно пов'язаний з порушеннями нормального видового складу мікробіоти молочної залози. Бактерії роду *Bifidobacterium* є важливим компонентом нормальної мікробіоти грудей і володіють протипухлинною активністю. Молекулярно-біологічні механізми взаємовпливу клітин РМЗ і представників мікробіоти ще остаточно не з'ясовані. **Метою** цього дослідження було розробити та оптимізувати експериментальну модельну систему культивування клітин РМЗ з *Bifidobacterium animalis in vitro*. **Матеріали та методи.** Об'єктами дослідження були РМЗ клітини людини ліній MCF-7, T47D та MDA-MB-231, а також живі та інактивовані високою температурою бактерії виду *Bifidobacterium animalis subsp. lactis (B. animalis)*. Кінетику росту та життєздатність *B. animalis* у присутності різних ліній клітин РМЗ людини та без них визначали методом турбідиметрії (OD_{600}) та паралельним висівом на елективне поживне середовище. Споживання глюкози і продукцію лактату біфідобактеріями оцінювали біохімічними методами. Життєздатність клітин РМЗ визначали стандартним колориметричним методом. **Результати.** Кінетика росту *B. animalis* у повному поживному середовищі DMEM відповідала стандартним параметрам кривої росту бактерій. Показники споживання глюкози і продукції лактату *B. animalis* підтверджують їхню фізіологічну метаболічну активність за досліджених умов росту. Присутність клітин РМЗ в модельній системі не впливала на тривалість фаз росту популяції клітин *B. animalis*, але сприяла збільшенню їх кількості. Статистично достовірне зменшення кількості живих клітин РМЗ усіх досліджених ліній спостерігали лише через 48 годин співкультивування з живими *B. animalis*. Для досягнення подібного ефекту пригнічення життєздатності пухлинних клітин необхідне 10—30 кратне збільшення кількості інактивованих високою температурою бактерій у порівнянні з живими. **Висновки.** Ідентифіковано найбільш оптимальні умови співкультивування клітин РМЗ людини та живих клітин *B. animalis in vitro*.

Ключові слова: рак молочної залози, Bifidobacterium, мікробіота, крива росту, pH средовища, культура клітин.