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Ye. Lukianova [1], O. Mushii [1, *], V. Bazas [2], O. Voronina [1], N. Cheremshenko [1]

[1] R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the NAS of Ukraine, Kyiv, Ukraine
[2] Kyiv City Clinical Oncology Center, Kyiv, Ukraine

* Correspondence: Email: sashamushy1@gmail.com

CYTOSTATIC EFFECTS AND MECHANISMS OF ACTION OF BACTERIAL LECTIN OF *BACILLUS SUBTILIS* IMV B-7724 ON BREAST CANCER CELL LINES OF DIFFERENT MOLECULAR SUBTYPES

Background. Lectins are carbohydrate-binding proteins with diverse biological activities, and they are increasingly recognized for their potential in cancer diagnostics and therapy. **Aim** of the study was to investigate the cytostatic and cytotoxic effects of the bacterial lectin of *Bacillus subtilis* IMV B-7724 on breast cancer (BC) cell lines of different molecular subtypes. **Materials and Methods**. Experiments were performed on the T47D (luminal A), MCF-7 (luminal B), and MDA-MB-468 (basal, triple-negative) cell lines. Cytotoxicity was assessed by crystal violet staining and LC_{50}/LC_{30} determination. Cytomorphological alterations were analyzed using the Romanowsky — Giemsa staining and ImageJ morphometry. A flow cytometry was applied to evaluate the cell cycle distribution and apoptosis (Annexin V/PI assay). The expression of proliferation and apoptosis regulators (Ki-67, p53, BCL-2, BAX) was examined by immunocytochemistry. **Results.** The lectin exerted dose-dependent cytotoxicity across all tested cell lines, with LC_{50} values ranging between 351—419 μg/mL. The morphological analysis revealed subtype-specific alterations, including cell rounding, nuclear condensation, and decreased cytoplasmic volume, indicative of the apoptotic process. The flow cytometry showed distinct cell cycle arrest patterns: S-phase accumulation in T47D, G2/M blockade in MCF-7, and combined S- and G2/M-phase arrest in MDA-MB-468. The viability assays confirmed a significant reduction of live cells, predominantly through late apoptosis and necrosis, with the strongest effect observed in T47D cells. The immunocytochemistry demonstrated a marked downregulation of Ki-67 and BCL-2, increased p53 expression, and BAX upregulation in MCF-7 cells, whereas T47D cells displayed a paradoxical BAX suppression despite the apoptotic features. **Conclusions.** Lectin of *B. subtilis* IMV B-7724 exerts multifaceted effects on BC cells of different molecular subtypes, including the suppression of cell proliferation, activation of apoptosis, and cell cycle modulation. The observed differences between the BC cell lines suggest that the lectin efficacy depends on both the molecular profile of the cells and the mode of apoptosis regulation (p53-dependent or p53-independent), warranting further studies to identify key targets.

Keywords: lectin, breast cancer, molecular subtype.

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Lectins are among the most intriguing and multifunctional groups of proteins, playing a key role in biological processes due to their ability to specifically recognize and bind carbohydrate structures [1]. Despite the discovery of them more than a century ago, they remain a subject of intensive research in molecular biology, biotechnology, and medicine. The uniqueness of lectins lies in their ability to interact with mono- and oligosaccharides on cell surfaces with high affinity and selectivity, without altering the covalent structure of substrates. This defines their involvement in such processes as cell adhesion, intercellular communication, signaling cascades, immune response, and the mechanisms of "self—non-self" recognition [2]. The binding sites of lectins are tuned to recognize certain carbohydrate configurations, enabling highly precise interactions within the cellular microenvironment. Importantly, lectins are widespread in nature: they have been found in plants, fungi, bacteria, viruses, animals, and humans. This universality underscores the evolutionary importance of carbohydrate—protein interactions [1].

Special attention should be paid to the role of lectins in the pathogenesis of malignant tumors. It is known that cell transformation is accompanied by a rearrangement of the membrane protein glycosylation, resulting in the formation of new glycoconjugates specific for tumors [3, 4]. Lectins expressed in the tumor microenvironment, as well as exogenous lectins, can recognize these structures and influence cell adhesive properties, invasion, and metastasis [5, 6]. Some of them have been identified as powerful immunomodulators capable of stimulating the cytotoxic activity of T lymphocytes or natural killer cells [7, 8]. This opens the way for their use as tumor biomarkers and potential therapeutic agents.

The biotechnological applications of lectins are also remarkably broad. They are used for glycoprotein analysis, biomolecule purification, cell sorting, and diagnostics [9]. Lectin microarrays have become an innovative tool for high-throughput glycosylation profiling in both normal and pathological states [10]. Furthermore, some lectins, owing to their ability to specifically bind oncoglycans, are applied as probes in histochemistry to detect malignant transformation [11].

At the current stage of scientific development, lectins are regarded not only as auxiliary research tools but also as promising agents for cancer immunotherapy. Their selectivity toward specific carbohydrate structures enables the development of targeted drug delivery systems, next-generation vaccines, and immunotherapeutic complexes [12]. At the same time, the potential toxicity of certain lectins should be mentioned as such that necessitates a careful control of their use [13, 14].

Thus, lectins represent a unique class of proteins that combine fundamental biological significance with applied potential. Studying the mechanisms of their action and interactions with glycoconjugates provides a deeper insight into the patterns of cell communication, immune responses, and the development of pathologies, particularly cancer and infectious diseases. Expanding the knowledge in this field will contribute not only to a deeper understanding of the biological foundations of life but also to the development of new approaches in the diagnosis and treatment of human diseases.

In our previous studies, we have demonstrated that the IMV B-7724 strain of *B. subtilis*, a spore-forming, aerobic, gram-positive bacterium, is a producer of lectin [15] that exhibited a high sugar-binding specificity toward N-acetylneuraminic and N-glycolylneuraminic acids [16]. At the same time, the full spectrum of effects, as well as the molecular targets of *B. subtilis* IMV B-7724 lectin in cells of different histogenesis, depending on their molecular profile, have not been thoroughly described.

Therefore, the aim of this study was to investigate the cytostatic effects and mechanisms of the action of the lectin of *B. subtilis* IMV B-7724 in breast cancer (BC) cells of different molecular subtypes.

Materials and Methods

Lectin isolation. The lectin was isolated from the culture medium of *B. subtilis* IMV B-7724 on the 4th day of culturing as described in [17]. The isolated lectin was freeze-dried at temperatures between −32 °C and +24 °C and stored as a powder at −20 °C.

Cell lines and lectin treatment. Three human BC cell lines of different molecular subtypes, T47D, MCF-7, and MDA-MB-468 [18, 19], were obtained from the Cell Bank of Human and Animal Tissue Lines, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the NAS of Ukraine. The cells were cultured in DMEM (with L-glutamine and glucose) (Sigma, USA), supple-

mented with 10% fetal bovine serum (BioWest, USA) at 37 °C in a humidified atmosphere with 5% CO_2. To assess the cytotoxicity of the lectin of *B. subtilis* IMV B-7724 in the BC cell lines of different malignancy grades, the lectin was added to the culture medium in concentrations ranging from 18.75 to 600 μg/mL. After 24 h of incubation, the cells were stained with crystal violet [20], and the dye adsorption was evaluated. Based on the obtained values, dose–response curves were plotted, and the LC_{30} and LC_{50} values were determined.

Cytomorphological and cytomorphometric analysis. For the immunocytochemical analysis, cells were grown on coverslips. Fixation was performed in a methanol/acetone solution (1:1) for 2 h at –20 °C. To evaluate the effects of the bacterial lectin on the BC cell lines, the cells were stained using the Romanowsky — Giemsa method [21]. At the next stage, microphotographs were taken and further used for morphometric measurements in ImageJ software. We measured area, perimeter, length, width, circularity, roundness, aspect ratio, and solidity for both nucleus and cytoplasm, as well as the nucleus-to-cytoplasm ratio. The circularity characterizes the approximation of the object's shape to the perfect circle (a value of 1 corresponds to the perfect circle; lower values indicate more elongated or irregular shapes). The roundness index reflects the degree of circularity, taking into account the length-to-width ratio. This aspect ratio represents the ratio of the maximum length to maximum width, allowing the assessment of cell or nuclear elongation. The solidity indicates the ratio of the object's area to the area of its convex hull, reflecting the presence of irregularities or indentations along the contour. These parameters enable the quantitative evaluation of changes in the shape and structural organization of cells and nuclei under the influence of the studied agents.

Flow cytometry. Apoptosis and cell viability were assessed using Annexin V staining combined with propidium iodide. For cell cycle analysis, cells were stained with propidium iodide after fixation in 70% ethanol and treatment with RNase A. The data were analyzed using FlowJo 10 software (Flow Jo, LLC,, USA). In addition, quantitative indices were calculated based on the distribution of cells in G0/G1, S, and G2/M phases. The proliferation index (PI) reflects the proportion of cells in the DNA synthesis and mitosis phases (S + G2/M), thus indicating the overall proliferative potential of the population. The G2+M/G0+G1 ratio shows the relative accumulation of cells in the mitotic phase compared with the resting phase, whereas the S/G0+G1 index indicates the extent of DNA replication phase accumulation relative to the resting phase.

Immunocytochemistry. Cell preparation and the protocol were performed according to the previous studies [22]. Monoclonal antibodies to Ki-67 (clone SP6, Diagnostic BioSystems, USA), p53 (clone SP5, Thermo Scientific, USA), BCL-2 (clone Bcl-2-100, Thermo Scientific, USA), and BAX (clone 2D2, Thermo Scientific, USA) were used in the dilutions recommended by the manufacturer. Visualization was performed using the Master Polymer Plus Detection System (Biocare Medical, USA). The expression was evaluated by counting immunopositive cells under an AxioScope microscope (Zeiss, Germany) at ×400 magnification. For semi-quantitative expression assessment, the H-score method was applied [23].

Statistical analysis. Statistical analyses were carried out using GraphPad Prism v.8.0 (GraphPad Software Inc., USA). Comparisons between two groups were performed using Student's *t*-test when the data distribution was normal, or the Mann — Whitney U test when the assumption of normality was not met. Results are expressed as M ± m (mean ± standard error of the mean) or as percentages. Differences were considered statistically significant at $p \leq 0.05$. The half-maximal lethal concentration (LC_{50}) was calculated using the linear regression analysis in Microsoft Excel, based on the equation of the best-fit straight line.

Results

The LC_{50} values of *B. subtilis* IMV B-7724 lectin for cells of both luminal subtype lines, T47D and MCF-7, were 357.64 μg/mL and 419.45 μg/mL, respectively. The corresponding value for the BC cells of the basal molecular subtype, MDA-MB-468, was 351.49 μg/mL.

The LC_{30} values for T47D and MCF-7 cells were 230.25 μg/mL and 251.80 μg/mL, respectively, whereas for MDA-MB-468 cells, the lowest value was observed at 204.01 μg/mL (Fig. 1). For further evaluation of the effects of lectin on the BC cell lines, the LC_{30} concentration was used.

The next stage involved the evaluation of cytomorphological changes in BC cells after exposure to *B. subtilis* IMV B-7724 lectin. In the T47D cells, lectin treatment reduced nuclear and cytoplasmic areas, de-

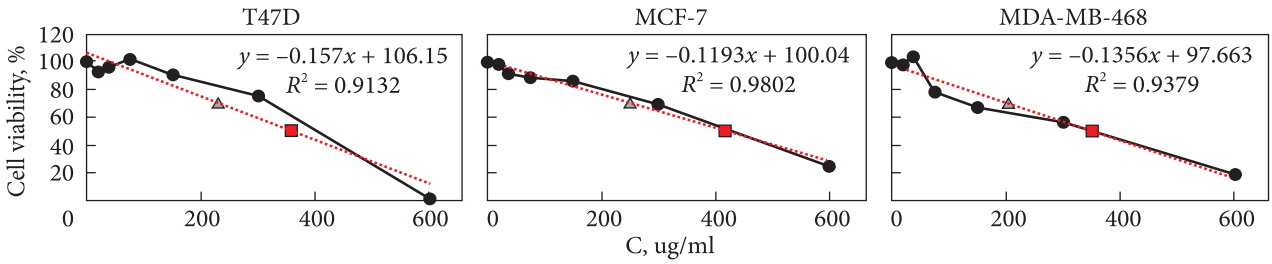


Fig. 1. Cytotoxicity of *B. subtilis* IMV B-7724 lectin toward the BC cells of different molecular subtypes: LC_{50} and LC_{30} values are marked with red and gray markers, respectively

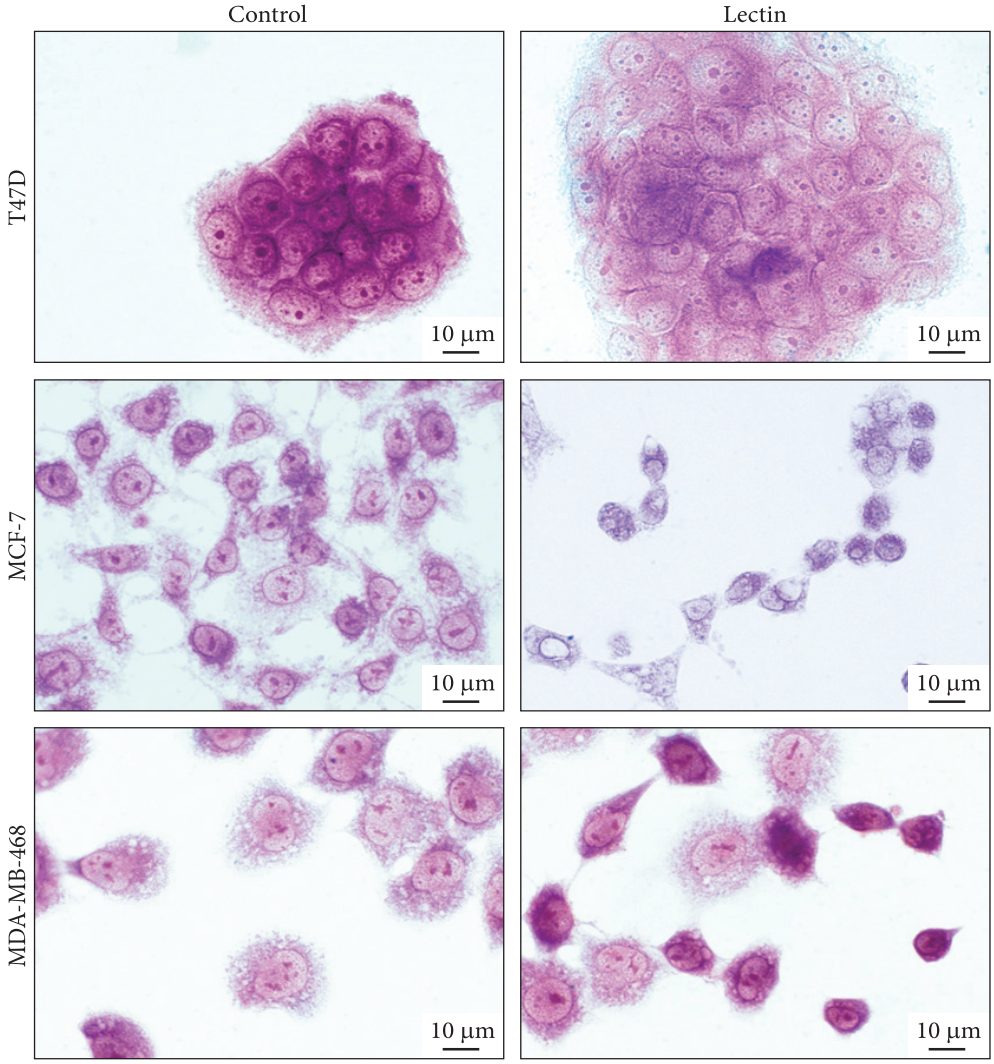


Fig. 2. Cytomorphological features of BC cells of different molecular subtypes after treatment with *B. subtilis* IMV B-7724 lectin. Romanowsky — Giemsa staining, ×1000

creased cell length and width, and increased circularity and solidity indices (Table 1). Morphologically, the luminal A subtype cells acquired a more rounded shape and polymorphism, while remaining organized in layers that did not disintegrate. The nuclei appeared lighter, with less intense basophilia and more dispersed chromatin, and in some cells, the nucleoli were less prominent (Fig. 2).

In the MCF-7 cells, lectin exposure was associated with a reduction in cytoplasmic and nuclear sizes, an increase in circularity and solidity indices, and a loss of protrusions and elongated morphology (Table 1, Fig. 2).

In the MDA-MB-468 cells of the basal molecular subtype, lectin treatment induced an increase in nuclear area and perimeter, accompanied by a

Table 1. **Morphometric parameters of the BC cell lines of the different molecular subtypes after treatment with *B. subtilis* IMV B-7724 lectin**

Cell compartment	Parameter Control	T47D		MCF-7		MDA-MB-468	
		Lectin	Control	Lectin	Control	Lectin	Control
Nucleus	Area, μm²	**121.42 ± 5.77**	**96.10 ± 4.83▼**	**78.97 ± 3.21**	**66.62 ± 3.63▼**	**113.80 ± 6.19**	**148.60 ± 6.32▲**
	Perimeter, μm	**39.60 ± 0.78**	**34.15 ± 0.74▼**	**32.0 ± 0.61**	**29.25 ± 0.71▼**	**38.01 ± 0.75**	**43.84 ± 0.92▲**
	Length, μm	**13.81 ± 0.27**	**12.02 ± 0.26▼**	**11.03 ± 0.19**	**10.04 ± 0.26▼**	**13.20 ± 0.26**	**15.30 ± 0.31▲**
	Width, μm	**10.92 ± 0.24**	**9.15 ± 0.22▼**	**8.821 ± 0.20**	**8.03 ± 0.21▼**	**10.60 ± 0.23**	**11.91 ± 0.27▲**
	Circle, a.u.	**0.944 ± 0.003**	**0.956 ± 0.003▲**	0.934 ± 0.004	0.926 ± 0.005	**0.952 ± 0.002**	**0.932 ± 0.004**
	Round, a.u.	**0.802 ± 0.010**	**0.826 ± 0.011▲**	0.799 ± 0.009	0.808 ± 0.012	0.807 ± 0.009	0.780 ± 0.011
	Aspect ratio, a.u.	1.29 ± 0.017	1.23 ± 0.016	1.273 ± 0.018	1.269 ± 0.023	1.26 ± 0.016	1.31 ± 0.018
	Solidity, a.u.	0.991 ± 0.003	0.993 ± 0.004	0.991 ± 0.0005	0.989 ± 0.0015	0.992 ± 0.0002	0.988 ± 0.0009
Cytoplasm	Area, μm²	**521.0 ± 33.80**	**361.5 ± 26.10▼**	**370.0 ± 23.48**	**225 ± 18.26▼**	504.0 ± 52.33	534.6 ± 29.04
	Perimeter, μm	**149.8 ± 4.85**	**120.9 ± 4.25▼**	**128.3 ± 3.98**	**88.29 ± 4.28▼**	**139.4 ± 4.39**	**119.6 ± 3.11▼**
	Length, μm	**36.10 ± 1.02**	**29.05 ± 0.98▼**	**30.1 ± 0.77**	**23.54 ± 0.84▼**	35.15 ± 0.99	34.69 ± 0.99
	Width, μm	**21.55 ± 0.81**	**17.10 ± 0.62▼**	**18.06 ± 0.60**	**14.82 ± 0.49▼**	**20.93 ± 0.87**	**23.77 ± 0.69▲**
	Circle, a.u.	**0.362 ± 0.011**	**0.541 ± 0.015▲**	**0.349 ± 0.012**	**0.559 ± 0.026▲**	**0.383 ± 0.010**	**0.582 ± 0.014▲**
	Round, a.u.	**0.612 ± 0.017**	**0.692 ± 0.016▲**	**0.609 ± 0.015**	**0.667 ± 0.018▲**	**0.608 ± 0.018**	**0.698 ± 0.014▲**
	Aspect ratio, a.u.	1.88 ± 0.061	1.71 ± 0.043	**1.769 ± 0.057**	**1.662 ± 0.067▼**	**1.812 ± 0.063**	**1.502 ± 0.036▼**
	Solidity, a.u.	**0.701 ± 0.017**	**0.803 ± 0.030▲**	**0.658 ± 0.011**	**0.787 ± 0.018▲**	**0.71 ± 0.009**	**0.827 ± 0.009▲**
Nucleus to cytoplasm ratio, a.u.		0.287 ± 0.012	0.309 ± 0.011	**0.248 ± 0.010**	**0.401 ± 0.032▲**	**0.272 ± 0.011**	**0.316 ± 0.011▲**

Note: ▲/▼— significant ($p < 0.05$) increase/decrease compared to the control group.

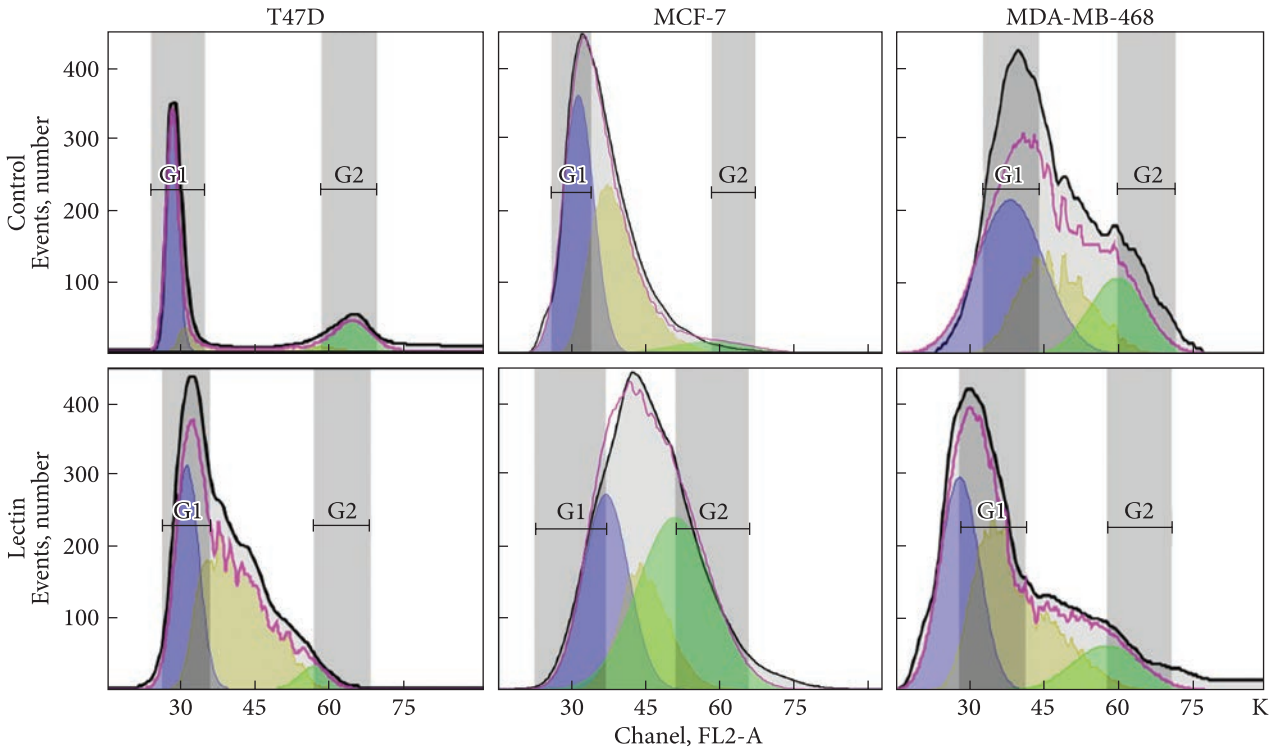


Fig. 3. Distribution of BC cells of different molecular subtypes across the cell cycle phases after exposure to *B. subtilis* IMV B-7724 lectin

moderate loss of roundness and a higher aspect ratio. The cytoplasm showed a reduced perimeter and an increased nucleus-to-cytoplasm ratio, indicating the condensation of cellular structures (Table 1). Morphologically, the cells became less polygonal and more compact, with marked cytoplasmic basophilia and more intense nuclear staining. In some cells, the chromatin appeared loosened and nucleoli enlarged, which is associated with the altered transcriptional activity (Fig. 2).

All the shifts above indicate the suppression of the functional activity and activation of the apoptotic processes in three cell lines under the action of the lectin, with the effect in T47D being less pronounced than in MCF-7 cells.

The analysis of the cell cycle phase distribution revealed pronounced differences between the control samples and cells incubated with lectin. For the T47D line, a decrease in the proportion of cells in G0/G1 from ~63% to ~36% was observed, along with a sharp increase in the number of cells in the S-phase (from ~14% to ~59%), indicating an accumulation of cells in the process of DNA replication and a less pronounced involvement of the G2/M phase.

Under the influence of lectin in the MCF-7 line, a decrease in the proportion of cells in the G0/G1 phase by almost 1.3 times and a sharp increase in their proportion in the G2/M phase (from ~6% to over 43%) were recorded, indicating a blockade of the mitotic transition. At the same time, a reduction in the number of cells in the S-phase was noted.

Similar cell cycle alterations after lectin treatment were observed in cells of the basal molecular subtype. As shown in Fig. 3 and Table 2, in the MDA-MB-468 line, on the contrary, a decrease in cells in G0/G1 and an accumulation in the S and G2/M phases (almost 1.5-fold) were recorded. This indicates a delay in the cell cycle at the stages of DNA synthesis and mitosis. The effect of lectin on BC cells of different malignancy grades was also accompanied by an increase in PI, which was most pronounced in the MDA-MB-468 line (≈1.4-fold).

In MCF-7 cells, lectin exposure caused a marked decrease in the number of cells in the G0/G1 phase with a simultaneous increase in the proportion of cells in the G2/M phase (Table 2). This was accompanied by nearly 40% reduction in the relative S/G0+G1 ratio and a sharp 10-fold rise in the G2+M/G0+G1 ratio, indicating a block of cells in the G2/M phase.

Cells of the luminal A subtype T47D demonstrated the opposite effect: the proportion of cells

in the G0/G1 phase dropped by 26.9%, while a strong accumulation of cells in the S-phase was observed (up to 59.4%). In this case, the S/G0+G1 ratio increased more than sevenfold (from 0.22 to 1.63), while the G2+M/G0+G1 ratio remained consistently low. This indicates an accumulation of cells in the S-phase at the stage of DNA replication under the lectin treatment.

In the MDA-MB-468 cells, the lectin exposure led to a decrease in the proportion of cells in G0/G1 (by 17%) with an increase in cells in the S-phase (by 10.7%) and the G2/M phase (by 6.4%). The S/G0+G1 and G2+M/G0+G1 ratios nearly doubled, indicating a simultaneous accumulation of cells in both the S and G2/M phases after the lectin treatment.

Overall, the lectin showed a subtype-specific effect on cell cycle regulation: in luminal B subtype MCF-7 cells, it induced a block at the G2/M phase, in luminal A subtype T47D cells, it caused a strong accumulation in the S-phase, while in basal subtype MDA-MB-468 cells, it produced a combined effect with disruption of transitions in both the S and G2/M phases. The increase in proliferation index (PI) under lectin treatment is likely associated with the cell cycle arrest in the S and G2/M phases as a consequence of damage and cytotoxic stress.

When assessing the cell viability after cultivation with lectin using Annexin V/PI staining, a significant decrease in the number of viable cells was observed in all tested lines (Fig. 4). In the T47D line, the proportion of viable cells decreased by 38,9%, which was accompanied by a sharp increase in necrotic cells (by 36%) and a moderate rise in late apoptosis (by 3%). The proportion of cells in early apoptosis under lectin exposure remained almost unchanged.

In the MCF-7 cells, lectin reduced the proportion of viable cells by 25.6%. This was accompanied by an increase in late apoptotic cells (by 5.91%) and a significant rise in necrotic cells (by 20.52%), as well as a decrease in cells at the early apoptosis stage (by 0.8%).

In the MDA-MB-468 cells, lectin reduced the proportion of viable cells by 16.2%. At the same time, a marked increase in late apoptosis (by 6.03%) and a moderate increase in necrotic cells (6.75%) were observed. Notably, in this triple-negative BC line, an increase in early apoptosis was recorded (by 3.43%), which may indicate a more gradual and moderate involvement of cells in the programmed cell death.

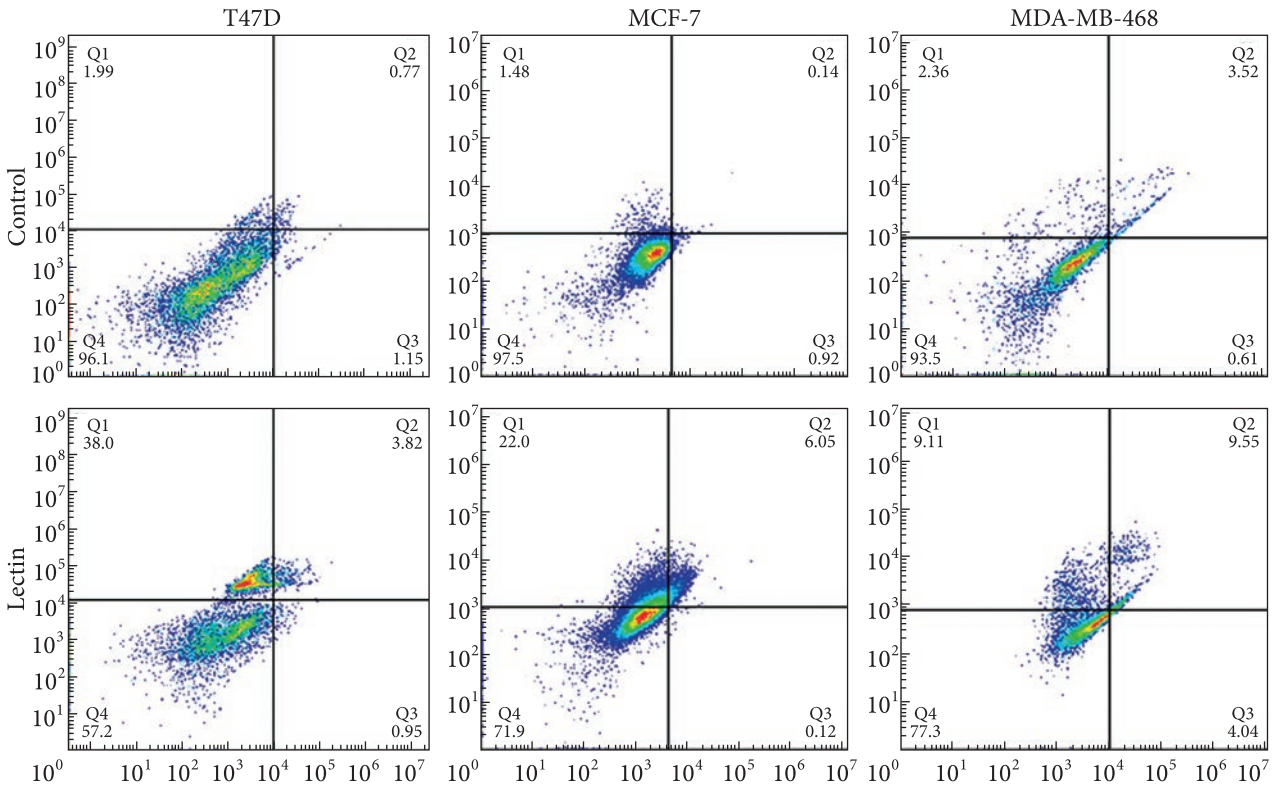
Thus, the lectin exposure led to a reduction in the BC cell viability primarily due to the induction of apoptosis and necrosis. The severity of the cytotoxic effect varied depending on the molecular subtype of the cells, being most pronounced in the T47D line, whereas MCF-7 and MDA-MB-468 cells displayed a more moderate response to lectin, likely related to their molecular-biological characteristics and greater resistance to cytotoxic stress. At the same time, it cannot be excluded that a longer lectin exposure may be required for these cell lines to develop a full cytotoxic effect.

At the final stage of the study, we evaluated the expression of the molecular markers of proliferation and apoptosis in the BC cell lines exposed to the lectin of *B. subtilis* IMV B-7724. As shown in Table 3, lectin treatment in all tested BC cell lines caused pronounced changes in the expression of key markers of proliferation and apoptosis. The

Table 2. **Distribution of BC cells of different malignancy grades across the cell cycle phases after exposure to *B. subtilis* IMV B-7724 lectin**

Cell cycle phases	T47D		MCF-7		MDA-MB-468	
	Control	Lectin	Control	Lectin	Control	Lectin
G0+G1	63.1	36.2 ▼	47.9	35.5 ▼	52.3	35.2 ▼
S	14.1	59.4 ▲	46.4	21.3 ▼	33.6	44.3 ▲
G2+M	22.8	4.4 ▼	5.7	43.2 ▲	14.1	20.5 ▲
PI	36.9	64.0 ▲	52.1	64.5 ▲	47.7	64.8 ▲
G2+M/G0+G1	0.36	0.12 ▼	0.12	1.22 ▲	0.27	0.63 ▲
S/G0+G1	0.22	1.63 ▲	0.97	0.60 ▼	0.64	1.26 ▲

Note: ▲/▼— statistically significant ($p < 0.05$) increase/decrease compared to the control.



Viability and death stages	T47D		MCF-7		MDA-MB-468	
	Control	Lectin	Control	Lectin	Control	Lectin
Live cells	96.1	57.2▼	97.5	71.9▼	93.5	77.3▼
Early apoptosis	1.15	0.95	0.92	0.12▼	0.61	4.04▼
Late apoptosis	0.77	3.82▲	0.14	6.05▲	3.52	9.55▲
Necrosis	1.99	38.0▲	1.48	22.0▲	2.36	9.11▲

Fig. 4. Changes in the percentage of cells in apoptosis and necrosis under the influence of the lectin of *B. subtilis* IMV B-7724 in the BC cell lines of different molecular subtypes. ▲/▼ — significant ($p < 0.05$) increase/decrease compared to the control

Ki-67 expression was significantly reduced by approximately 3—5-fold compared to control. An increase in the expression of p53, associated with the stress response and apoptosis, was observed, indicating the activation of the cellular damage response cascade. The strongest increase in this protein's expression was recorded in the MCF-7 cells (1.66; $p < 0.05$).

Regarding the anti-apoptotic protein BCL-2, its expression was significantly reduced in all cell lines, approximately 3—8-fold, indicating a weakening of cellular protective mechanisms in re-

Table 3. **Changes in the expression of apoptosis- and proliferation-regulating proteins in BC cells of different molecular subtypes under the influence of *B. subtilis* IMV B-7724 lectin**

Cell line	Group	Ki-67	p53	Bcl-2	Bax
T47D	Control	89.81 ± 2.40	108.84 ± 5.44	134.79 ± 8.21	167.90 ± 13.24
	Lectin	6.97 ± 0.94 ▼	133.49 ± 6.26 ▲	34.02 ± 2.51 ▼	36.82 ± 7.72 ▼
MCF-7	Control	291.0 ± 15.4	92.76 ± 1.54	108.74 ± 2.92	132.81 ± 2.84
	Lectin	76.83 ± 4.48 ▼	153.82 ± 3.42▲	13.91 ± 2.58 ▼	169.90 ± 2.91 ▲
MDA-MB-468	Control	249 ± 2.7	188.32 ± 17.46	204.98 ± 23.44	52.98 ± 6.52
	Lectin	29.21 ± 2.11 ▼	45.18 ± 4.80 ▼	2.58 ± 0.89 ▼	45.65 ± 5.36

Note: ▲/▼— significant ($p < 0.05$) increase/decrease compared to the control group.

sponse to the activation of apoptotic cascades. At the same time, the expression of the pro-apoptotic protein BAX increased in MCF-7 cells (by 27.93%; $p < 0.05$), corresponding to the activation of the apoptotic pathway under the influence of the lectin of *B. subtilis* IMV B-7724. The most pronounced reduction (over 4.56-fold) in BAX expression in T47D cells is likely due to rapid protein degradation, its suppression through intracellular signaling networks activated by lectin exposure, or alterations in the functional activity of the mutant p53 form characteristic of this cell line [24, 25]. Overall, these findings indicate that the lectin significantly inhibits BC cell proliferation and shifts the balance toward apoptosis by altering the expression ratios of p53, BAX, and BCL-2.

Discussion

We demonstrated that the lectin of *B. subtilis* IMV B-7724 exerts a pronounced cytotoxic effect on BC cells. However, the nature and intensity of the response varied significantly depending on the biological characteristics of the cell lines. The cytotoxic effect was accompanied by morphological and functional changes, cell cycle disruption, reduced proliferative activity, and activation of apoptosis. We established that the LC_{50} values indicate relatively high sensitivity of both hormone-dependent and triple-negative cells, yet morphological and functional analyses revealed that the most pronounced changes occurred in luminal B subtype MCF-7 cells, which exhibit the highest proliferative activity values among the available in vitro BC models [26]. This may be explained by the differing levels of membrane glycoconjugate expression, which are potential lectin targets. The hormone-dependent cells are known to have a more complex glycosylation phenotype, which may underlie their vulnerability to lectin-mediated cytotoxicity [27].

The effect of *B. subtilis* IMV B-7724 lectin was mediated by the decreased expression of Ki-67 and BCL-2, increased levels of p53 and BAX, and induction of late apoptosis. The analysis of the apoptosis markers revealed the differences in the mechanisms underlying the cytotoxic effects of *B. subtilis* IMV B-7724 lectin. In the MCF-7 and MDA-MB-468 cells, the effect was associated with increased p53 expression and decreased BCL-2 levels, consistent with the classical apoptotic pathway. In contrast, the T47D cells exhibited a paradoxical reduction in the expression of the anti-apoptotic protein BAX, despite the morphological features of apoptosis, suggesting the BAX-independent apoptotic mechanisms [28] or a shift toward the necrotic cell death [29].

Thus, *B. subtilis* IMV B-7724 lectin exerts multifaceted effects on BC cells of different molecular subtypes, including suppression of cell proliferation, activation of apoptosis, and cell cycle modulation. The observed differences between the BC cell lines suggest that the lectin efficacy depends on both the molecular profile of the cells and the mode of apoptosis regulation (p53-dependent or p53-independent), warranting further studies to identify the key targets.

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Є. Лук'янова [1], О. Мушій [1], В. Базась [2], О. Вороніна [1], Н. Черемшенко [1]

[1] Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького НАН України, Київ, Україна
[2] Київський міський клінічний онкологічний центр, Київ, Україна

ЦИТОСТАТИЧНІ ЕФЕКТИ І МЕХАНІЗМИ ДІЇ БАКТЕРІАЛЬНОГО ЛЕКТИНУ *BACILLUS SUBTILIS* IMV B-7724 НА КЛІТИНИ РАКУ МОЛОЧНОЇ ЗАЛОЗИ РІЗНОГО МОЛЕКУЛЯРНОГО ПІДТИПУ В СИСТЕМІ IN VITRO

Стан питання. Лектини — це білки, що зв'язують вуглеводи та мають широкий спектр біологічної активності; останнім часом вони дедалі більше розглядаються як перспективні агенти для діагностики та терапії раку. У даному дослідженні вивчали цитостатичні та цитотоксичні ефекти бактеріального лектину *Bacillus subtilis* IMV B-7724 на клітинних лініях раку молочної залози (РМЗ) різних молекулярних підтипів. **Методи.** Експерименти проводили на клітинах ліній T47D (люмінальний A), MCF-7 (люмінальний B) та MDA-MB-468 (базальний, тричі-негативний). Цитотоксичність оцінювали методом фарбування клітин кристалічним фіолетовим з визначенням LC_{50}/LC_{30}. Цитоморфологічні зміни аналізували на препаратах, зафарбованих за Романовським — Гімзою, та подальшою морфометрією в ImageJ. Для оцінки розподілу клітинного циклу та апоптозу (Annexin V/PI-аналіз) застосовували проточну цитофлуориметрію. Експресію маркерів проліферації та регуляторів апоптозу (Ki-67, p53, Bcl-2, Bax) досліджували методом імуноцитохімії. **Результати.** Лектин виявив дозозалежну цитотоксичність у всіх досліджуваних клітинних лініях із значеннями LC_{50} у межах 351—419 мкг/мл. Морфологічний аналіз показав підтип-специфічні зміни, зокрема округлення клітин, конденсацію ядер та зменшення об'єму цитоплазми, що вказує на апоптичні процеси. Проточна цитофлуориметрія виявила різні варіанти блокади клітинного циклу: накопичення клітин у S-фазі (T47D), зупинку у фазі G2/M (MCF-7) та комбіновану блокаду S- і G2/M-фаз (MDA-MB-468). Тести на життєздатність підтвердили достовірне зниження кількості живих клітин переважно за рахунок пізнього апоптозу та некрозу, причому найсильніший ефект спостерігався у T47D. Імуноцитохімія продемонструвала значне зниження експресії Ki-67 і Bcl-2, підвищення рівня p53 та активацію BAX у клітинах MCF-7, тоді як T47D характеризувалися парадоксальним зниженням BAX попри наявні ознаки апоптозу. **Висновки.** Лектин *Bacillus subtilis* IMV B-7724 індукує підтип-специфічну цитотоксичність клітин РМЗ шляхом пригнічення проліферації, порушення клітинного циклу та активації апоптозу. Його диференційна активність, особливо виражена у люмінальному B та потрійно-негативному підтипах, підкреслює його потенціал як селективного протипухлинного агента та кандидата для комбінованих терапевтичних стратегій. Подальші дослідження необхідні для з'ясування молекулярних мішеней та оптимізації застосування цього лектину в імунотерапії раку.

Ключові слова: лектин, рак молочної залози, молекулярні підтипи.