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FEATURES OF THE SYNTHESIS OF EXTRACELLULAR CYTOTOXIC LECTIN *BACILLUS SUBTILIS* IMV B-7724, DEPENDING ON THE CULTIVATION CONDITIONS IN THE LABORATORY FERMENTER

The level of oxygen mass transfer (K_V) is an important parameter influencing the growth rate of aerobic microorganisms and the synthesis of metabolites. It is mainly determined by the agitation and the aeration rates in the fermenter. ***Aim of the study****. To study changes in pH, optical density (OD), and hemagglutinating (lectin) activity (HAA) of culture fluid (CF) of Bacillus subtilis strain IMV B-7724, a producer of extracellular cytotoxic lectin (ECL), during its cultivation in a laboratory fermenter at different agitation and aeration rates as well as to determine and compare the HAA, carbohydrate specificity, and cytotoxic properties of the corresponding samples of the preparation isolated from CF.* ***Methods.*** *Batch antifoam-free fermentations were performed by culturing the strain in the modified Gause medium with galactose in two identical lab-scale fermenters with a working volume of 2.5 L at 37 °C for 48—72 h according to three fermentation variants. Variant 1: n — 400 rpm for the whole cultivation, the air supply to the CF — through a sparger at 0.1 vvm until the 39th h with further gradual decrease, K_V — 4.2 ± 0.3 g $O_2 \cdot L^{-1} \cdot h^{-1}$. Variant 2: n — 400 rpm for the first 24 h, then a gradual decrease to 200 rpm, air supply — through a sparger at 0.1 rpm for the first 12 h, followed by its switching into the fermenter free space, corresponding K_V — from 4.2 ± 0.3 to 0.3 ± 0.1 g $O_2 \cdot L^{-1} \cdot h^{-1}$. Variant 3: n — 400 rpm and air supply to the fermenter free space during the whole cultivation, K_V — 4.0 ± 0.3 g $O_2 \cdot L^{-1} \cdot h^{-1}$. A number of biological*

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properties of strain CF and isolated lectin samples were evaluated by biochemical, spectrophotometric, immunological, and culture methods. Statistical analysis was performed using Student's t-test. ***Results****. The maximum increase in the OD of CF relative to the initial values (28 and 21-fold) at the end of the period of the rapid growth of the strain (at 9th h), the μ_{max} values of 0.33 and 0.41 h^{-1}, and pH not lower than 6.7 and 6.3 units were observed for fermentation variants 1 and 2, respectively. In the case of variant 2, the HAA of CF reached 32 hemagglutinating units (HAU), and the samples isolated from it had a lectin activity of 512 ± 64 HAU, whereas for variant 1 such values were lower:16 and 32 ± 8 HAA, respectively; carbohydrate specificity of preparations to bovine submandibular gland mucin was the same, i.e. 0.2 ± 0.1 mg/mL. In contrast to the above, a slower increase in the OD of the CF, a decrease in μ_{max}, and significant acid formation (15-fold at the 9th h, 0.25 h^{-1}, and pH decrease to 5.8 units, respectively) were observed for variant 3; in this case, the level of HAA of CF was minimal (2—4 HAU) and was absent in the corresponding isolated samples . The probable reason for such differences was the limited mass transfer in the CF due to the isolating effect of the foam layer on its surface formed as a result of intensive agitation.* ***Conclusions.*** *The rapid growth of the strain and an increase in the HAA of CF were observed during cultivation in a lab-scale fermenter by maintaining the maximum level of oxygen mass transfer with air supply into the CF through a sparger until the maximum OD was reached and the subsequent gradual decrease in the specified level during further cultivation started.*

Keywords: *Bacillus subtilis IMV B-7724, laboratory fermenter, oxygen mass transfer, extracellular cytotoxic lectin, hemagglutinating activity, carbohydrate specificity, cytotoxic properties.*

Obtaining biologically active substances of microorganisms capable of specific effects on immunogenesis, immune response, and tumor growth control remains very relevant. In particular, long-term studies of extracellular sialospecific lectins of saprophytic aerobic bacteria of the *Bacillus* genus have shown that they are characterized by a selective effect on tumors of various origins, are inducers of natural gamma-interferon synthesis [1—4], as well as active inhibitors of adsorption and reproduction of influenza, herpes, hepatitis C, and HIV viruses [5—8]. The presence of such unique properties opens up broad prospects for the use of these substances in biology and medicine, in particular for the development of new biotherapeutic agents to improve the effectiveness of anticancer therapy. It was found earlier that the maximum increase in the lectin activity of culture fluid (CF) of various *Bacillus* species occurs at the end of the logarithmic growth phase of cultures, and the further repeated increase in this activity is associated with cell autolysis and the subsequent release of intracellular lectins into the CF, which allowed the author [9] to consider them as secondary metabolites.

A previous study of the biological activity of the CF of *Bacillus subtilis* strain IMV B-7724 showed the presence of a lectin-like substance with pronounced cytotoxic and cytolytic effects on tumor cells [3]. Accordingly, the highest HAA (128 hemagglutinating units (HAU)) was observed in CF samples obtained within 2—5 days, whereas the maximum yield of the preparation (80 mg/L) with HAA of 2048 HAU and cytotoxic activity (IC — 95.0 ± 2.0%) was obtained on the 4th day of strain growth. Further study of the chemical composition, carbohydrate specificity, and physicochemical properties of the extracellular cytotoxic lectin (ECL) of strain IMV B-7724 revealed that the obtained bacterial lectin was a glycoprotein with a molecular mass of 18—20 kDa with the highest affinity to N-acetylneuraminic and N-glycolylneuraminic acids, high thermal stability, resistance to changes in pH, and long-term storage [4]. In this work, CF for subsequent lectin isolation was obtained by growing the strain in shaking flasks, which is usually used in laboratory studies, but the results obtained in this way cannot be directly extrapolated to the possible productivity of a similar process on a larger scale, usually performed in fermenters (bioreactors) with a stirrer. This is due to large differences in the effects of physical and biological factors on the growth and

biosynthesis of microorganisms in the first and second cases. Thus, the successful implementation of production on a large scale requires the maintenance of appropriate parameters and regimes optimized for the growth of the particular microorganism and its synthesis of the target product. For this purpose, a detailed preliminary study of these processes under controlled conditions in low-volume lab-scale fermenters is needed. In view of the above, the **aim** of this work was to study the growth peculiarities of *Bacillus subtilis* IMV B-7724, changes in pH and HAA of CF during batch cultivation of the strain in laboratory fermenters differing in agitation speed, method of air supply and its flow rate as well as to investigate carbohydrate specificity and cytotoxic properties of extracellular lectin preparations obtained under different fermentation conditions.

Materials and methods. The saprophytic strain *B. subtilis* IMV B-7724 was used in this work as a producer of ECL from the collection of the R.E. Kavetskyi Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine [2]. The inoculum was obtained by growing the strain on Hromyko agar medium (meat-peptone agar: wort agar, 1:1) at 37 °C for 24 h. To obtain a liquid inoculum, we used the Gause liquid medium modified for the synthesis of lectins [10]. The medium was sterilized under a steam pressure of 0.75 kg / cm^2 for 30 min, cooled to room temperature, its pH was set at 7.0 ± 0.1 using a 10% solution of KOH, aseptically, and inoculated with the inoculum removed from agar. The inoculated liquid medium was added to 100 mL in 750 mL shake flasks, which were shaken on a shaker (n — 160 rpm) for 4 days at 37 °C. The grown liquid culture was used as a liquid inoculum.

Experiments to study the effect of fermentation time and conditions on the growth and synthesis of extracellular lectin were performed by simultaneous batch cultivation of strain B-7724 in two identical laboratory fermenters Biotec FL 103 (Biotec AB, Stockholm, Sweden) of a total volume of 4 L each (Fig.1).

Each fermenter was a baffled cylindrical glass vessel with a working volume of 2.5 L, having an internal diameter of 160 mm and a height of 210 mm with a single impeller mounted on the shaft. Four baffles with a width of 20 mm and a

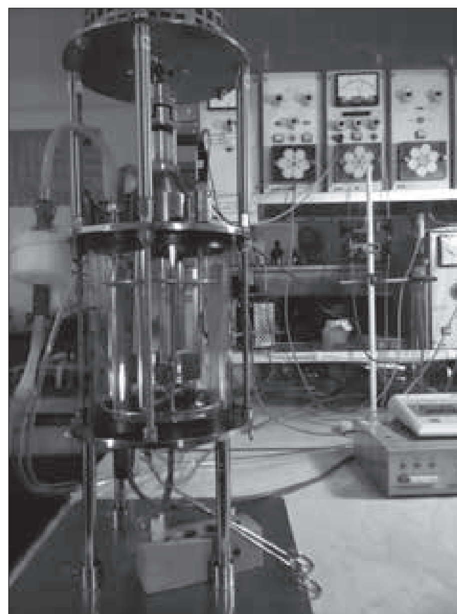


Fig. 1. Laboratory fermenter FL-103

height of 134 mm, which was close to the height of the liquid filling the tank, were placed perpendicular to the vessel. Each of the systems was equipped with an enlarged six-bladed stainless steel impeller for agitation designed and made by us (blade with a height of 30 mm and a width of 24 mm located on the upper side of perforated disk with a diameter of 90 mm). The impeller disk was located at a distance of 46 mm from the vessel bottom. The sparger, a short-length vertical pipe with a single 0.9 mm diameter hole for aeration air outlet on its conical top, located under the impeller disk at a distance of 6 mm, was fixed in the bottom.

Sterile fermenters were filled with the above sterile cooled medium in the amount of 2.35 L, its pH was adjusted to 7.0 ± 0.1 as described above, and then heated to 37 °C. 0.15 L of liquid inoculum (6% by volume) was added to the heated medium, and fermentation started. All operations were performed aseptically. The fermentation was carried out at 37 °C and monitored by a temperature probe, which was controlled by circulating chilled water.

The efficiency of application of 3 variants of fermentation, which differed in the method of air supply for aeration of the CF, and the applied regimes of agitation speed and air flow rate were investigated. Thus, the agitation speed was varied from 200 to 400 rpm; the air flow rate in fermentation systems was constantly monitored by rotameters. Its values of 0.05, 0.10, and 0.25 $L \cdot min^{-1}$ were used when the air was supplied through the sparger and of 0.10, 0.40, and 3.00 $L \cdot min^{-1}$ — when it was supplied into the free space of the fermenters above the CF surface.

Oxygen mass transfer rate (K_V), g $O_2 \cdot L^{-1} \cdot h^{-1}$, which characterizes the aeration system and the intensity of oxygen dissolution in the liquid medium, was determined previously by the specific oxidation rate of sodium sulfite solution for each case of air supply and agitation rate by the conventional sulfite method [11]. Its use provides data only to compare the oxygen uptake rate of sodium sulfite solution under conditions of aeration and agitation that are identical to the cultivation conditions.

In particular, according to ***variant 1 of fermentation***, the air was supplied into the CF through a sparger (1st method of air supply) at the flow rate of 0.25 $L \cdot min^{-1}$, a corresponding specific value — 0.1 vvm (volume of air supplied to the volume of liquid per minute) from the beginning to 39 h of cultivation. Air flow was further reduced gradually: to 0.10 $L \cdot min^{-1}$ from the 39th to the 42nd h and to 0.05 $L \cdot min^{-1}$ — from the 42nd to the 48th h, the end of fermentation. The agitation speed was maintained at 400 rpm throughout the fermentation period. The K_V value was 4.2 ± 0.3 g $O_2 \cdot L^{-1} \cdot h^{-1}$.

In ***variant 2 of fermentation***, aeration air was also supplied into the CF through a sparger at a flow rate of 0.25 $L \cdot min^{-1}$, but only during the period of intensive bacterial strain growth (first 12 hof cultivation). Air was further passed only into the free space of fermenters (2nd method of air supply) at a flow rate of 0.40 $L \cdot min^{-1}$ from the 12th to the 24th h and of 0.10 $L \cdot min^{-1}$ — from the 24th h to the 72nd h (the end of cultivation). The following agitation speed regimes were used: 250 rpm — from the beginning to the 3rd h; 370—400 rpm — from the 3rd to the 24th h and with a further gradual decrease to 350, 300, 250, and 200 rpm from the 24th, 30th, 48th, and 54th huntil the end of fermentation, respectively. The above values of air flow and agitation speed corresponded to the following values of K_V: 1.0 ± 0.1; 4.2 ± 0.3; 2.3 ± 0.2; 1.2 ± 0.1; 0.5 ± 0.1, and 0.3 ± .1 g $O_2 \cdot L^{-1} \cdot h^{-1}$.

According to ***variant 3 of fermentation***, aeration air was supplied only into the free space of the fermenters, above the surface of the CF (2nd method of air supply) at a flow rate of 3.00 $L \cdot min^{-1}$ and maintained agitation speed of 400 rpm throughout the fermentation period, 48 h. The K_V value was 4.0 ± 0.3 g $O_2 \cdot L^{-1} \cdot h^{-1}$.

The foam that constantly accumulated on the

surface of the CF when air was supplied through a sparger (during the whole fermentation for variant 1 and within the first 12 h for variant 2) was destroyed by jets of sterile air continuously injected at a rate of 13—18 L·min^{-1} through devices specially designed and installed by us inside the fermenters. Fermentation was repeated twice for each variant, and no chemical defoamers were used to control the foam level.

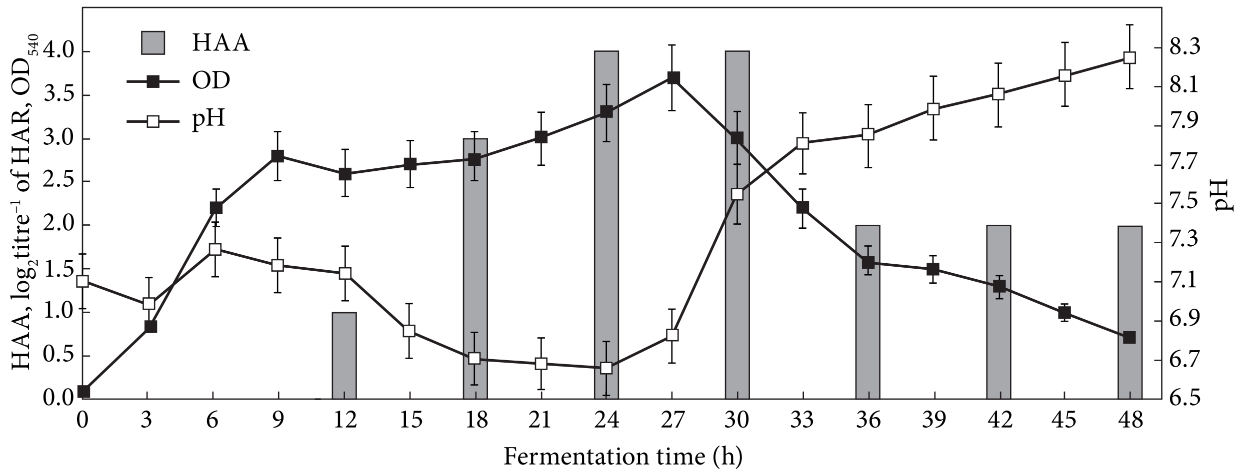
Metabolic and growth activities of the strain were evaluated by changes in active acidity (pH, ionometer I-160MI) and optical density (OD, photocolorimeter KFK-2, λ — 540 nm, *l* — 3 mm) of CF samples (sampling — every 3 h of fermentation). Based on the obtained data, the maximum specific growth rate of the culture (μ_{max}) in the exponential growth phase (from the 3rd to the 6th h of fermentation) was calculated. The rate of extracellular lectin biosynthesis was estimated by the HAA of CF supernatants. For this purpose, samples of CF were taken from the fermenters every 6 h, starting from the 12th h of fermentation and centrifuged (2500 g, 15 min). The obtained supernatants were immediately frozen and stored at —17 °C.

HAA was determined by the hemagglutination reaction (HAR) method in a U-bottom microplate using a series of two-fold dilutions of the CF supernatant samples immediately after thawing or a 1% solution of dry lectin preparation with a 2% suspension of trypsin-treated and glutaraldehyde-fixed rabbit erythrocytes at room temperature. HAA was expressed as the reciprocal of the highest dilution (titer) at which agglutination was still observed (HAR titer^{-1}) or $\log_2$ titer^{-1} of HAR. For example, 2HAU = 1$\log_2$ titer^{-1} of HAR when it was still observed for the 1st dilution; 4HAU = 2$\log_2$ titer^{-1} of HAR when it was still observed for the 2nd dilution; 8HAU = 3$\log_2$ titer^{-1} of HAR, when it was still observed for the 3rd dilution, etc. A 2% suspension of erythrocytes in buffered physiological solution in the absence of the test substance was used as an autoagglutination control [12].

The process of isolation and purification of the active substance of the lectin preparation from the CF, as well as determination of HAA, carbohydrate specificity, and cytotoxic activity against Ehrlich ascitic adenocarcinoma cells of extracellular lectin preparations obtained under these conditions were performed as described in [3, 4]. Data were analyzed statistically using variation statistics methods [13].

Results. The dynamics of changes in pH, OD, and HAA of CF during three variants of fermentation of the strain B-7724 in lab-scale fermenters was studied (Fig. 2 a, b, c). Batch cultivation of the strain was characterized by the presence of two or three periods of acid formation, the intensity and duration of the decrease in the CF, pH of which depended on the chosen variant.

Fermentations according to variants 1 and 2. Characterized by a less level of acid formation in general. In particular, the first period lasted from the beginning to 3 h, and the second period lasted from 12 to 24 h of fermentation (Fig. 2 a, b). The minimum pH values of CF observed in the first and second acidification periods for the 1st—2nd fermentation variants were 7.0/6.8 units, and 6.7/6.3 units, respectively. In addition, a third, insignificant decrease in the CF pH values from 7.01 to 6.77 was observed between the 33rd and 39th h of fermentation for variant 2. The described periods of decreasing pH could be due to germination of a certain part of spores with the formation of vegetative cells, which was accompanied by some increase in the metabolic rate and the increase in the optical density of the culture liquid. Generally, these pH values were close to neutral, which is considered optimal for the active growth of these bacteria. The increase in OD of CF was most rapid for the first 9 hours for the 1st and 2nd fermentation variants and reached 2.8 and 3.1 units (an increase of 28 and 20.7 times the initial values) at the end of this period; μ_{max} values were 0.33 and



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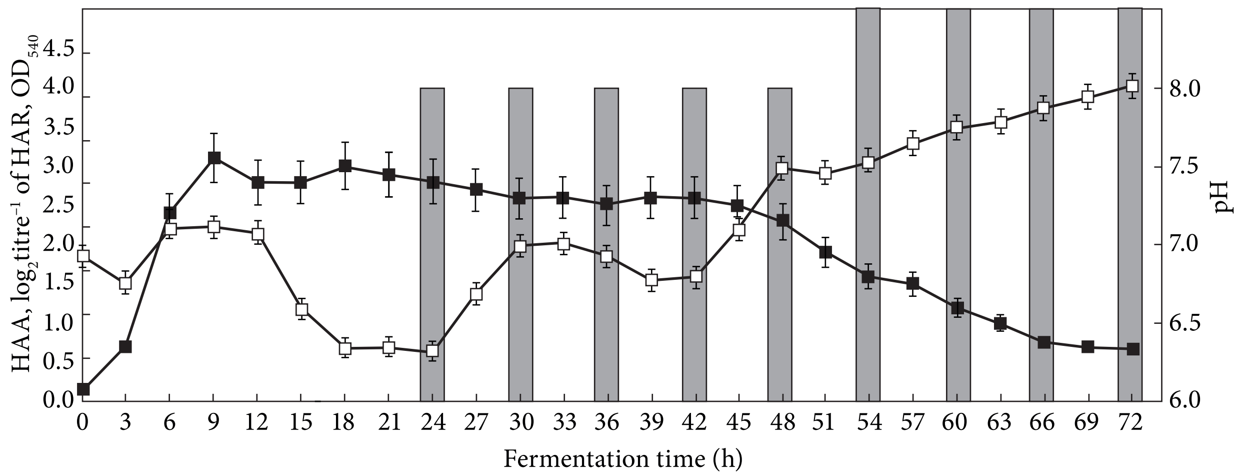

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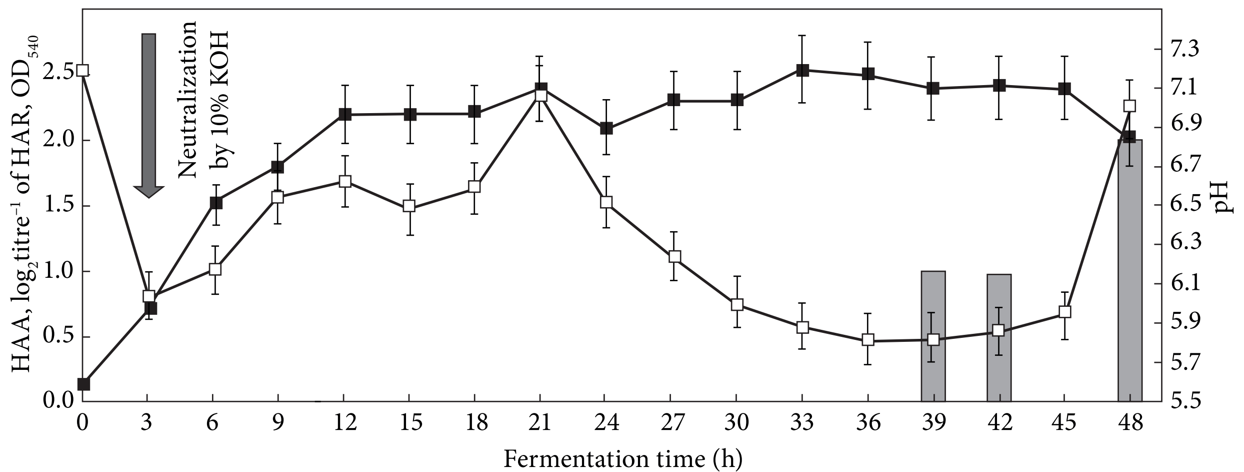

c

Fig. 2. The dynamics of changes in pH, OD, and HAA of CF of strain B-7724 in laboratory fermenters: *a* —variant 1 of fermentation; *b* —variant 2 of fermentation; *c* —variant 3 of fermentation

0.41 h^{-1}, respectively. The level of HAA of CF reached $4\log_2$ HAR $titer^{-1}$ at the 24th h but subsequently decreased to $2\log_2$ HAR $titer^{-1}$ from the 36th h until the end of cultivation for variant 1 (Fig. 2 a), and for variant 2 it increased to $5\log_2$ HAR $titer^{-1}$ from the 54th h to the completion (Fig. 2 — b).

Fermentation according to the 3rd variant. Acidification was more intense in general, and the duration of its first period was relatively longer (from the beginning to 6 h of fermentation). In particular, pH was decreased to a suboptimal value of 5.6 for the 3.5—4th h of growth (Fig. 2c), after which it was corrected once to 6.3 as described above, the moment of neutralization is indicated by the arrow. Further, the pH of CF slowly increased from 6 h to 21 h and reached 7.1, after which a second, longer period of acid formation began, with a gradual decrease to 5.8 at the 36th h. Finally, pH of CF again increased over the last 12 h to 7.0 at the end of fermentation (48 hours). The increase in the OD of the CF was not as rapid as for variants 1 and 2 of fermentation and was only 1.8 at the 9th h, which corresponded to a 15-fold increase from the initial value, μ_{max} was 0.25 h^{-1}, and the period of intense growth was extended to 12 h.

HAA and carbohydrate specificity are the main biological characteristics of lectin preparations. We investigated these characteristics on experimental samples of ECL preparations obtained by culturing strain B-7724 in laboratory fermenters under different conditions (Table 1.)

As listed in the table, the HAA of samples of preparations obtained using fermentation variants 1 and 2 was 32 ± 8 and 512 ± 64 units, respectively, while the sample of the preparation isolated from CF during cultivation of the producer by variant 3 showed no HAA.

The most important characteristic of sialospecific lectin is its carbohydrate specificity expressed as a minimum concentration of a particular carbohydrate — bovine submandibular gland mucin, which interacts with lectin and inhibits HAR. Thus, inhibition of HAR was observed at a minimum concentration of this carbohydrate of 0.2 ± 0.1 mg/mL for both lectin preparations isolated from CF during the producer cultivation according to variants 1 and 2 of fermentation.

Samples of the lectin preparation isolated from CF of the strain obtained according to fermentation variants1 and 2 were also characterized by the presence of cytotoxic activity against tumor cells Table 1. Thus, IC_{50} (the substance concentration at which 50% of the target cells were killed) was 0.43 and 0.34 mg/mL, respectively.

Discussion. As known, various strains of aerobic spore-forming bacteria of *Bacillus subtilis* species are widely used for the industrial production of a number of enzymes, proteins, etc. due to their ability to release a significant amount of various extracellular metabolites into the culture medium [14]. The presence among them of compounds with pronounced surface-active properties, such as low molecular weight lipopeptides [15—17] causes intensive foaming of CF when growing this microorganism on liquid nutrient media with deep aeration, which significantly complicates the

Table 1. **Bioactivity characteristics of experimental samples of ECL preparations obtained by culturing strain B-7724 in laboratory fermenters under different conditions**

Cultivation conditions of the strain in laboratory fermenters	HAA,* $titer^{-1}$ HAR	Carbohydrate specificity,** mg/mL	Cytotoxic activity,*** IC_{50}, mg/mL
Variant 1 of fermentation	32 ± 8	0.2 ± 0.1	0.43 ± 0.01
Variant 2 of fermentation	512 ± 64	0.2 ± 0.1	0.34 ± 0.01
Variant 3 of fermentation	0	0	0

Note: * — for lectin solution at a concentration of 1 mg/mL; ** — to mucin of bovine submandibular gland; *** — against Ehrlich ascites adenocarcinoma cells *ex vivo*

cultivation process. In particular, this harmful phenomenon leads to significant losses of cells and their metabolites due to the removal of foamed CF from the bioreactor, increases the risk of contamination, requires a significant decrease in the working volume of the bioreactor and, consequently, in the productivity of the process. The traditional way to control foam levels during aerobic cultivation is to select and use effective inert chemical defoamers, and this approach is almost always applied in cases of fermentations using *B. subtilis* in bioreactors. However, it is known that the addition of some defoamer reduced the rate of oxygen transfer into the medium and could have a toxic effect on the physiology of microbial culture cells, as well as lead to equipment fouling during subsequent processing of CF [18].

We have not found any evidence for the cultivation of *B. subtilis* in fermenters to produce extracellular lectins in the available literature except our previous work [19], and the results presented here are a continuation of research in this direction, which was initiated for the first time. It should be noted that the first experience with the cultivation of another strain *B. subtilis* IMV B-7014, a producer of extracellular lectin with antiviral properties in the above lab-scale fermenters, was rather problematic. In particular, the results of a series of cultivations carried out with a filling ratio of 0.62 at the beginning of the process and a minimum aeration flow rate of 0.25 L·min^{-1} (0.1 vvm) without a defoamer showed that the loss of CF exceeded half the initial volume for the entire fermentation period (21 h) due to the emissions of its foamed fraction. The significantly higher content of all protein components in the foam and lectin activity compared to the corresponding values for CF in the fermenter, as we found, was an additional reason to consider these losses highly undesirable. When various chemical defoamers were used, foam emissions were stopped, but their presence in CF prevented the formation of a lectin precipitate while it was further salting out with ammonium sulfate from the supernatant [20].

We tested the use of two reagent-free methods to prevent foam overflow during fermentation. The first method was to provide additional airflow to remove the foam as mentioned above; it effectively cleaned the CF surface from foam and eliminated foam emissions but led to accelerated water evaporation and some biomass being carried away by the exhaust airflow outside the fermenter. Another obvious consequence of this application was a significant improvement in the aeration intensity and mass transfer rate of CF.

The second method was to apply air only over the surface of CF in the state of intensive agitation for its aeration (see fermentation variant 3, materials, and methods). The formed foam layer was characterized by a thickness of about 20—30 mm, which did not increase under these conditions and allowed us to avoid emissions during the whole period of cultivation. However, as mentioned above, in this case, the rates of increase in OD of CF and μ value were markedly lower compared to the values given above for fermentation variants 1 and 2. In our opinion, the probable reason for these differences was the limitation of the mass transfer due to the insulating effect of the foam layer, which prevented the contact of the CF surface with the air fed into the free space of the fermenter. As a consequence, the oxygen requirement for the fast-growing culture in the logarithmic growth phase was not fully satisfied despite the high agitation rate. The decrease in pH to values of 5.8—5.9 at 33—36 h of cultivation, caused, in our opinion, by a significant accumulation of acid metabolites or dissolved CO_2 in CF, can be considered as another confirmation of the mass transfer limitation under such conditions.

Oxygen is the main limiting factor of aerobic fermentation processes because its solubility and diffusion coefficient are low, while the con-

sumption coefficient, on the contrary, is high. Therefore, the level of mass transfer is mainly determined by the transfer of oxygen from the medium to microbial cells and the removal of metabolic products from them. The agitation speed and aeration rate in the fermenter are the main parameters influencing the level and balance of oxygen sorption — carbon dioxide desorption processes. Taking into account the high growth rate of *B. subtilis* at 37 °C on the one hand, and on the other hand, the fact that the bioreactor design and its regime for the cultivation of fast-growing bacteria ($\mu > 0.2\ h^{-1}$) must ensure an oxygen transfer rate of 4—5 kg $O_2 \cdot m^{-3} \cdot h^{-1}$ or more [21], we maintained this parameter not less than 4.0 g $O_2 \cdot L^{-1} \cdot h^{-1}$ by setting the maximum agitation speed during the active growth period of strain IMV B-7724.

It is important to note that the K_V values we determined previously by the sulfite method for the mode with the maximum speed of agitator rotation (400 rpm) did not differ for the deep and surface methods of aeration air input into the fermenter (1st and 2nd method of air supply, respectively) and were 4.2 ± 0.3 and 4.0 ± 0.3 g $O_2 \cdot L^{-1} \cdot h^{-1}$, as indicated above. This can be explained by the fact that the working solution of sodium sulfite in distilled water was able to absorb oxygen at a significantly higher rate due to the capture of part of the air flow fed into the free space of the fermenter under vigorous turbulence conditions (400 rpm) because it did not form a foam layer, unlike *B. subtilis* CF. An additional confirmation of this explanation is the simple recalculation of the given airflow rate (0.25 $L \cdot min^{-1}$ or 0.1 vvm) fed through the sparger to the corresponding specific oxygen flow rate of approximately 1.6 g $O_2 \cdot L^{-1} \cdot h^{-1}$, which is significantly lower compared to the K_V value obtained by the sulfite method.

As noted above, the maximum HAA was observed in CF samples obtained within 2—5 days of cultivation of strain IMV B-7724 in shaking flasks, whereas the highest HAA and OD of CF in the laboratory fermenter for variant 1 were observed much earlier — at 24—30 h and at 27 h of fermentation, respectively. But further cultivation led to a rapid decrease in OD of CF and its GAA from 36 h. The reason for this phenomenon, in our opinion, could be the process of intensive autolysis of the bacterial cells and partial loss of the CF lectin activity caused by its excessive oxygenation as a result of both application of additional vigorous air injection for foam control and maximum agitation speed during the whole period of cultivation.

In addition, the high agitation rate can cause significant shear stress, which negatively affects cell activity in the fermentation system, and this is considered as another possible reason for the decrease in HAA of CF in the second half period for variant 1 of fermentation. Thus, a study of variability in surfactin production under different agitation speeds during cultivation of *B. subtilis* Y9 in a 5-L fermenter showed that the volumetric mass transfer rate and oxygen-uptake rate were positively correlated with volumetric surfactin productivity; the highest production of surfactin was observed at 400 rpm, but it was reduced at the highest agitation speed of 500 rpm, especially after spore appearance and remained low until the end of the culture [16].

At the same time, the decrease in OD of CF was not as sharp when the air supply was stopped simultaneously both through the sparger and for foam destruction after reaching the maximum OD of CF and with further gradually reducing agitation speed and switching air supply into the free space of fermenter (2nd variant 2 of fermentation). Importantly, an increase in HAA of CF up to 5$\log_2$ HAR $titer^{-1}$ was observed over the last 18 h of fermentation, whereas this value decreased in variant 1, as shown above.

Thus, the results obtained show that the use of deep aeration by feeding air into the CF through a sparger with simultaneous constant foam destruction until the maximum OD of the CF was reached, ensured the rapid growth

of the *B. subtilis* strain IMV B-7724 in a laboratory scale fermenter and achieved HAA of 16—32 HAU. The HAA titers of the experimental samples of the ECL preparation were 32 ± 8 and 512 ± 64 HAU for variants 1 and 2, respectively, and had a carbohydrate specificity of 0.2 ± 0.1 mg/ml. Cultivation of the strain according to variant 3 of fermentation with aeration air supply only over the CF surface to avoid foam emission significantly slowed down the growth rate of the strain and was accompanied by a significant decrease in pH, probably due to a decrease in mass transfer resulting from the insulating effect of the foam layer formed as a result of intensive agitation. Under such conditions, HAA of CF was detected at a minimum level of 4 HAU only at the end of cultivation, and it was absent in the corresponding preparation samples.

This paper presents the results of the first study of the growth and ECL synthesis of strain *B. subtilis* IMV B-7724 on a lab scale. We believe thatit demonstrates for the first time the successful approach of using sterile air jets as a reagent-free method for controlling foam emissions during fermentation, since we have not found any published confirmation of such an application in bioreactors of any size. The results presented here suggest the need for further studies on the optimization of the cultivation parameters for the growth of this strain in a laboratory fermenter in order to obtain a preparation of extracellular cytotoxic lectin with high activity and specificity values.

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ОСОБЛИВОСТІ СИНТЕЗУ ПОЗАКЛІТИННОГО ЦИТОТОКСИЧНОГО ЛЕКТИНУ *BACILLUS SUBTILIS* ІМВ В-7724 В ЗАЛЕЖНОСТІ ВІД УМОВ КУЛЬТИВУВАННЯ У ЛАБОРАТОРНОМУ ФЕРМЕНТЕРІ

Рівень масообміну кисню є важливим параметром, від якого залежить інтенсивність росту аеробних мікроорганізмів і синтезу метаболітів. Він визначається, головним чином, швидкістю перемішування та рівнем аерації у ферментері. **Мета.** Дослідити зміни значень pH, оптичної густини (ОГ) та гемаглютинуючої (лектинової) активності (ГАА) культуральної рідини (КР) штаму *Bacillus subtilis* ІМВ В-7724 — продуцента позаклітинного цитотоксичного лектину (ПЦЛ) при його вирощуванні в лабораторних ферментерах із різною інтенсивністю перемішування і аерації. Визначити і порівняти ГАА, вуглеводну специфічність і цитотоксичні властивості відповідних зразків препарату, виділених із КР продуцента. **Методи.** Періодичні ферментації без використання хімічних піногасників проводили шляхом культивування штаму у модифікованому середовищі Гаузе із галактозою у двох однакових лабораторних ферментерах робочим об’ємом 2,5 л при 37 °С протягом 48—72 год згідно 3-х варіантів. 1-й варіант: n — 400 об/хв протягом усього терміну, подача повітря аерації у КР через барботер на рівні 0.1 $л \cdot л^{-1} \cdot хв^{-1}$ до 39ї год культивування з подальшим поступовим зменшенням, K_V — 4.2 ± 0.3 г $O_2 \cdot л^{-1} \cdot год^{-1}$; 2-й варіант: n — 400 об/хв до 24ї

год, потім — зі ступінчастим зниженням до 200 об/хв, подача повітря аерації — через барботер на рівні 0.1 $л \cdot л^{-1} \cdot хв^{-1}$ до 12 ї год, і з переключенням її у вільний простір ферментера у подальшому, K_V — від 4.2 ± 0.3 до 0.3 ± 0.1 г $O_2 \cdot л^{-1} \cdot год^{-1}$; 3-й варіант: n — 400 об/хв, подача повітря у вільний простір ферментера протягом усього терміну, K_V — 4.0 ± 0.3 г $O_2 \cdot л^{-1} \cdot год^{-1}$. Ряд біологічних властивостей КР штаму та виділених зразків лектину оцінювали біохімічними, спектрофотометричними, імунологічними та культуральними методами. Статистичний аналіз проводили за допомогою t-критерію Стьюдента. **Результати.** Максимальне збільшення ОГ КР відносно початкових значень (у 28 і 21разів) в кінці періоду швидкого росту штаму (на 9 год), значення μ_{max} (0,33 та 0,41 $год^{-1}$) та показник pH КР не нижче 6.7 та 6.3 одиниць відмічали для 1-го і 2-го варіантів ферментації, відповідно. ГАА КР досягала рівня 32 гемаглютинуючих одиниць (ГАО), а виділені з неї зразки препарату мали лектинову активність 512 ± 64 ГАО для 2-го варіанту, тоді як для 1-го варіанту ці показники були низькими — відповідно 16 ГАО і 32 ± 8 ГАО; вуглеводна специфічність препаратів до муцину підщелепної залози бика була однаковою і становила 0,2 ± 0,1 мг/мл. На відміну від вищенаведеного, уповільнення зростання ОГ КР, зменшення μ_{max} і суттєве кислотоутворення (відповідно у 15 разів на 9-ту год, 0,25 $год^{-1}$, зниження pH до 5,8) спостерігали для варіанту 3; за цих умов рівень ГАА у КР був мінімальним (2—4 ГАО), а у відповідних виділених зразків препарату — відсутнім. Ймовірною причиною таких відмінностей було обмеження рівня масообміну у КР внаслідок ізолюючого впливу шару піни на її поверхні, утвореної внаслідок інтенсивного перемішування. **Висновки.** Швидкий ріст штаму і збільшення ГАА КР при культивуванні у лабораторному ферментері відбувались в умовах максимального рівня масообміну з подачею повітря аерації у КР через барботер до досягнення максимального значення ОГ КР і наступного поступового обмеження масообміну при подальшому культивуванні.

Ключові слова: *позаклітинний цитотоксичний лектин, Bacillus subtilis IMB B-7724, лабораторний ферментер, масообмін, гемаглютинуюча активність, вуглеводна специфічність, цитотоксичні властивості.*