

---

<https://doi.org/10.15407/dopovidi2020.08.081>  
UDC 615.324-022.51:579.8-026.81]; 58.02:577.114.7

**L.A. Safronova, S.I. Voychuk, O.S. Brovarska**

Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Kyiv  
E-mail: safronova\_larisa@ukr.net

## **Influence of nanobiocomposites on the exopolysaccharide matrix of *Bacillus* strains**

*Presented by Academician of the NAS of Ukraine V.S. Pidgorskyi*

*The effect of nanobiocomposites of carrageenan and galactomannan — perspective prebiotics on the formation of the extracellular polysaccharide complexes of *Bacillus amyloliquefaciens* subsp. *plantarum* UCM B-5139 and UCM B-5140 probiotic strains is studied. Analysis of data showed that bacterial strains had differences in the content of various sugar residues within their exopolysaccharide matrices. Both compounds, carrageenan and galactomannan, and their nanocomposites showed a potency to change the content of the extracellular matrix of bacilli probiotic cells. The effect of these compounds depended, presumably, on the natural structural properties of polysaccharides of these strains. However, the synthesis of extracellular polysaccharides by *Bacillus* probiotic strains was not blocked in the presence of the investigated nanocomposites.*

*The obtained results indicate the possible dual (direct and indirect) action of polysaccharides and their nanocomposites in case of their use as the prebiotic components of synbiotic preparations: through the direct biological action on the properties of the mammalian epithelial cells or gut microflora, and indirectly through the changes of properties of the extracellular polysaccharide matrix of probiotic strains.*

**Keywords:** *probiotics, Bacillus strains, prebiotics, nanobiocomposites, polysaccharide matrix.*

For the normal functioning of organism, a balanced composition of the intestinal microbiota is necessary. Beneficial intestinal bacteria activate systemic and local immunity, participates in the metabolic processes of an organism and maintains their balance, play an important role in protecting an organism from pathogenic microorganisms. Probiotics, prebiotics, and synbiotics are used to correct the gut microbiota composition [1]. Extensive researches of the last few decades have demonstrated the health benefits of probiotic preparations.

In recent years, there has been an active tendency in the world to create complexes, based on probiotics and prebiotics, synbiotic preparations that have multifunctional properties. Probiotics are live microorganisms that confer a health benefit on the host, when administered in adequate amounts [2]. Prebiotics are preparations of non-microbial origin, which selectively

---

Цитування: Safronova L.A., Voychuk S.I., Brovarska O.S. Influence of nanobiocomposites on the exopolysaccharide matrix of *Bacillus* strains. *Допов. Нац. акад. наук Укр.* 2020. № 8. С. 81–91. <https://doi.org/10.15407/dopovidi2020.08.081>

stimulate the growth and activity of beneficial intestinal microflora [3]. More often, carbohydrates are used as prebiotics, which are not capable to be digested by macroorganism.

Most of the synbiotic preparations described in the literature contain the probiotics representatives of the indigenous intestinal microflora, which are the lactic acid bacteria most frequently [1, 3]. However, as components of complex preparations, it is advisable to use probiotic strains of transient microflora, which include bacteria of the genus *Bacillus*. Bacilli are characterized by a wide range of biological activity synthesizing antibiotics, enzymes, amino acids, polysaccharides and other compounds that possess an immunomodulatory activity as well [4, 5].

The interest in bacilli, as a part of probiotic compositions, grew significantly over the last 15 years. This is due to the accumulation of scientific data demonstrating their preventive and therapeutic efficacy in diseases of the gastrointestinal tract, disorders of the immune status and general metabolism [4].

There are a lot of various compounds that can be used for the construction of new synbiotic preparations. Among them, the complexes of carrageenan and galactomannan (both are polysaccharides) with organomineral ammonium-magnesium phosphate are of great interest, since they may possess broader biological activities. Carrageenan and galactomannan and, to a greater extent, their nanocomposites, stimulate growth and biological activity of lactic acid bacteria, of probiotic strains of *Bacillus amyloliquefaciens* subsp. *plantarum* UCM B-5139 and UCM B-5140, and, in addition, exhibit immunomodulatory properties [6, 7]. Previously, we showed that the *Bacillus* strains UCM B-5139 and UCM B-5140 themselves synthesize a number of biologically active substances including polysaccharides, which can be key players that determine their probiotic activity. It is known nothing about the impact of carrageenan and galactomannan on the content of the polysaccharides synthesized by bacterial cells. Therefore, the aim of this study was to evaluate the possible effect of carrageenan and galactomannan and their nanobiocomposites on the structural properties of the extracellular polysaccharides of bacterial cells.

**Materials and Methods. Bacteria.** *Bacillus amyloliquefaciens* subsp. *plantarum* strains UCM B-5139 and UCM B-5140 from the Ukrainian Collection of Microorganisms were used in the study. These bacteria are the components of the probiotic “Endosporin” [5]. Bacteria strains were cultivated on synthetic medium [7].

**Nanobiocomposites.** The organomineral nanobiocomposites of ammonium-magnesium phosphate with carrageenan and galactomannan were used in the study. These compounds were synthesized in the Favorskii Irkutsk Institute of Chemistry (Siberian Branch, Russian Academy of Sciences) and were kindly presented for research. To enhance the prebiotic activity of polysaccharides, the ammonium magnesium phosphate was incorporated into their structure [7]. The final organomineral nanocomposites contained forms of carbon, nitrogen, phosphorus, sulfur, and magnesium, which are readily accessible for microorganisms.

**Cytotoxic and proliferative potential of compounds.** To evaluate any potential cytotoxic and/or proliferative activity of the compounds on the eukaryotic cells, we used yeast *Saccharomyces cerevisiae* Y-517 from the Ukrainian Collection of Microorganisms as a model. Yeast cells were grown in the YPD medium until log-phase (to have a population of actively dividing cells at the various stages of the cell cycle) and were washed and resuspended in distilled water before treatment with compounds of known concentrations. Yeast cells were treated for 2 h that is close to the longevity of the cell division cycle, which is 2.5 h. The water suspensions of compounds were

used for the yeast treatment to avoid the possible co-influence of various nutrient medium components. After a series of dilutions, cells were plated on the YPD-agar plates and cultivated until the visible colonies appeared (48 h). The ability of compounds to inhibit or stimulate the yeast cells' growth was used as an indicator of cytotoxic and proliferative activities, correspondingly.

**Cultivation with compounds.** To study the influence of the nanobiocomposites on the bacteria exopolysaccharide matrix structure, the compounds were added to the medium at concentrations 2.5 g/L and 10 g/L as a single source of carbon. Bacteria were cultivated with compounds for 24 h. The control cells were cultivated in the synthetic medium with glucose as a carbon source.

**Lectin-gold binding analysis.** To evaluate the sugar residues that present in the exopolysaccharide matrices of the bacteria, the aliquots of bacteria suspensions were dropped onto a clean Parafilm surface, and the microscopy copper grids covered with a formvar film were put on them. After 15 min, the grids were washed three times (5 sec each) in water and put on the drops of various lectin-gold suspensions for 1 h. Grids were repeatedly washed three times with water to wash out unbind lectins. Then the grids were dried in open air for 30 min and analyzed with transmission electron microscopy. The number of gold particles per square unit ( $\mu\text{m}^2$ ) was determined for each sample, and this number was used to determine semiquantitatively the portion of each sugar residue in the extracellular matrix of bacteria.

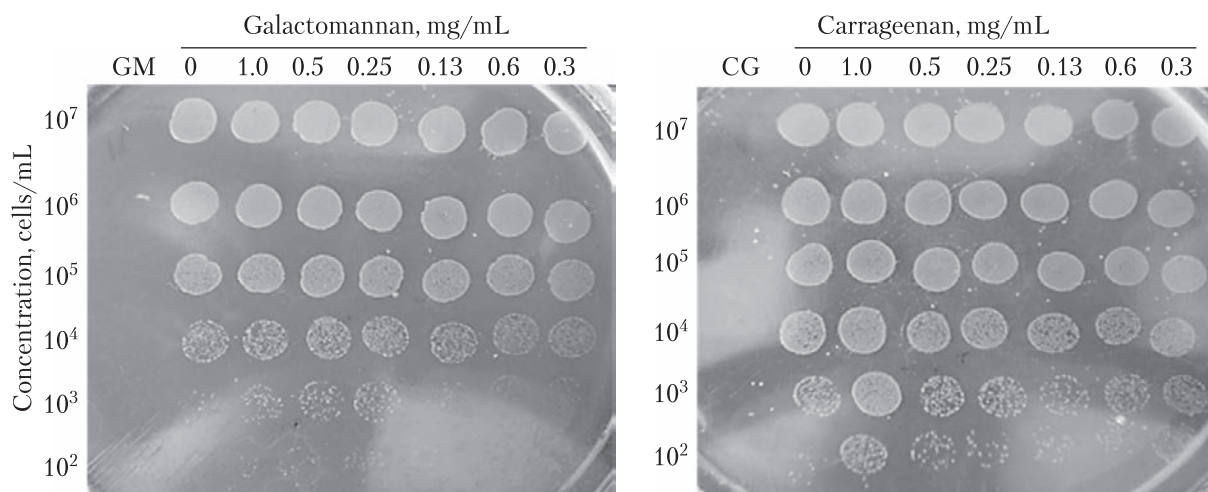
Lectin-gold complexes used in the current study were: "PHA" is a lectin from *Phaseolus vulgaris* (red kidney bean) highly specific to galactose, *N*-acetyl-*D*-glucosamine, and mannose, "WGA" is a wheat germ agglutinin with a high specificity to *N*-acetyl-*D*-glucosamine and sialic acid, "LCA" is a *Lens culinaris* agglutinin highly specific to *D*-mannose and *D*-glucose. All lectin-gold complexes were produced by "Lectinotest" (Ukraine). Solutions of lectin-gold complexes were centrifuged at 1000 rpm for 10 min before use to sediment any aggregates and only supernatant fraction was used for analysis according to the manufacturer recommendations.

**Mucopolysaccharides staining.** Ruthenium red was used to visualize mucopolysaccharides within the extracellular matrix. Bacteria cells adhered to the surface of the formvar film on the copper microscopy grids were put on the drops of ruthenium red and left for 1 h. After that, the grids were washed three times with water and dried in open air for half an hour.

**Electron microscopy.** Transmission electron microscope JEM-1400 (Jeol, Japan) was used to visualize the lectin-gold particles bound to the exopolymeric matrix of bacteria cells and for other visual observations. The accelerating voltage was 80 kV and magnification was  $\times 10\text{k} - 80\text{k}$ . The digital images were prepared.

**Image analysis.** Digital images were processed and analyzed with the help of ImageJ 1.50i, the free software developed by the National Institute of Health, USA and available at <https://imagej.nih.gov/ij/>. The number of gold particles per square unit ( $\mu\text{m}^2$ ) was determined for each sample.

**Statistics.** All samples of bacteria cultivated with the compounds were prepared in triplicate. Each sample was prepared at least in triplicate. At least five images were prepared for each sample. Each image was processed with image analysis software and the number of gold particles per  $\mu\text{m}^2$  that reflects semi-quantitatively the amounts of each sugar residue in the extracellular matrix of bacteria was determined. The mean value and standard deviation were determined. The significance of the difference between the mean values was determined by the *t*-test for in-



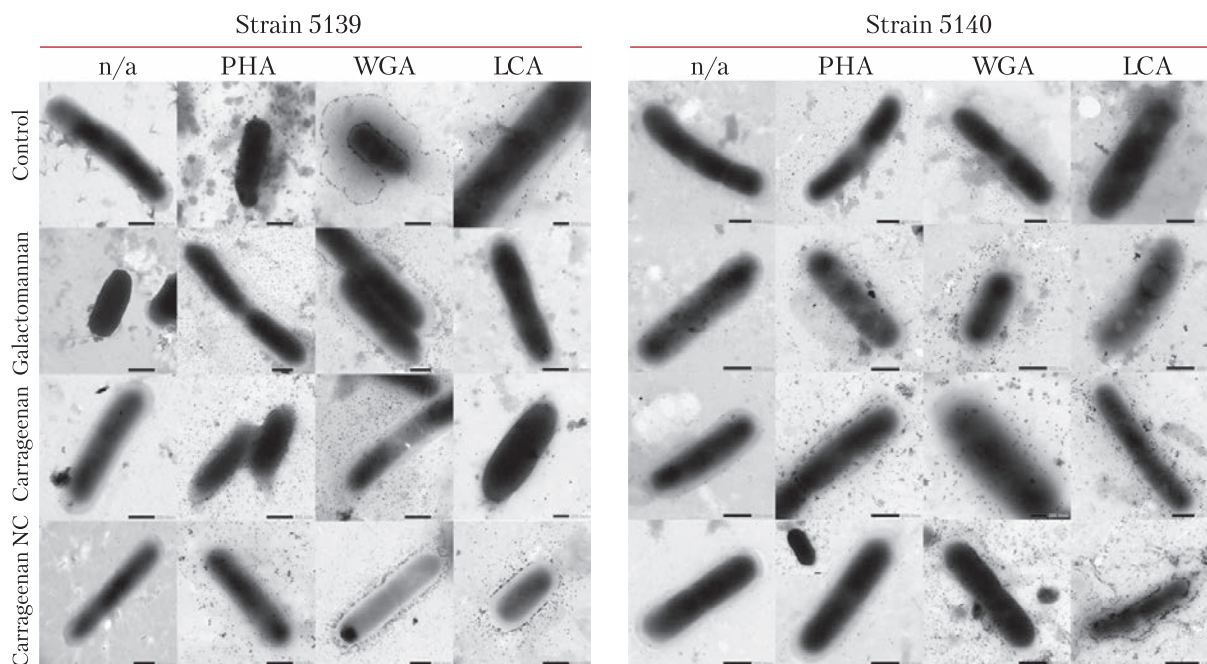
**Fig. 1.** Viability of *Saccharomyces cerevisiae* Y-517 cells after 2 h treatment with galactomannan and carrageenan. “GM” and “CG” are, correspondingly, serial dilutions of galactomannan (1 mg/mL) and carrageenan (1 mg/mL) to check their clearness

dependent samples with the help of Statistica version 10 (StatSoft Inc. (2011), USA, www.statsoft.com). The  $p \leq 0.05$  was chosen as a measure of the significance of the differences.

**Results and discussion. Cytotoxicity study of nanocomposites of galactomannan and carrageenan.** The studied nanocomposites of galactomannan and carrageenan at concentrations 0.03-1.0 mg/mL showed no cytotoxic activity against *Saccharomyces cerevisiae* cells: the radial growth of the colonies, their shape and size did not change as a result of the cell interaction with the nanocomposites for 2 h (Figure 1). Moreover, the induction of cell division was marked at high concentrations of the compounds.

Considering that these nanocomposites did not exhibit cytotoxic action against the eukaryotic model organism *Saccharomyces cerevisiae*, we can suggest a high potency of non-toxicity of these substances for eukaryotic cells in general. This, in turn, points to the potential prospects of these compounds as ingredients of various probiotic preparations. Therefore, at the next stage, the ability of these substances to influence the synthesis of exopolymers by the bacilli cells was studied.

**Influence of nanocomposites of galactomannan and carrageenan on the synthesis of exopolymers of *B. amyloliquefaciens* subsp. *plantarum* UCM B-5139 and UCM B-5140.** Lectins bind with specific markers like colloidal gold or fluorochromes applied for the quantitative and qualitative analysis of an extracellular polysaccharide matrix [8, 9]. We used lectins-gold particles to evaluate the effect of carrageenan (CG) and galactomannan (GM) and their nanocomposites (CG-Nano and GM-Nano) on the exopolysaccharide layers that surround the bacterial cells. There are two main aspects that can be evaluated with the help of lectins: (i) quantity of specific sugar residues, that provides a general information about the gross quantities of specific bonds, and (ii) the difference between the binding capacities of lectins with the common specificities, that provides the most interesting information since lets to predict the polysaccharide structure. We used lectins with well-established sugar-binding specificities (LCA, WGA, and PHA). The LCA binds to mannose and glucose residues and has an additional specificity to *N*-acetyl-*D*-glu-



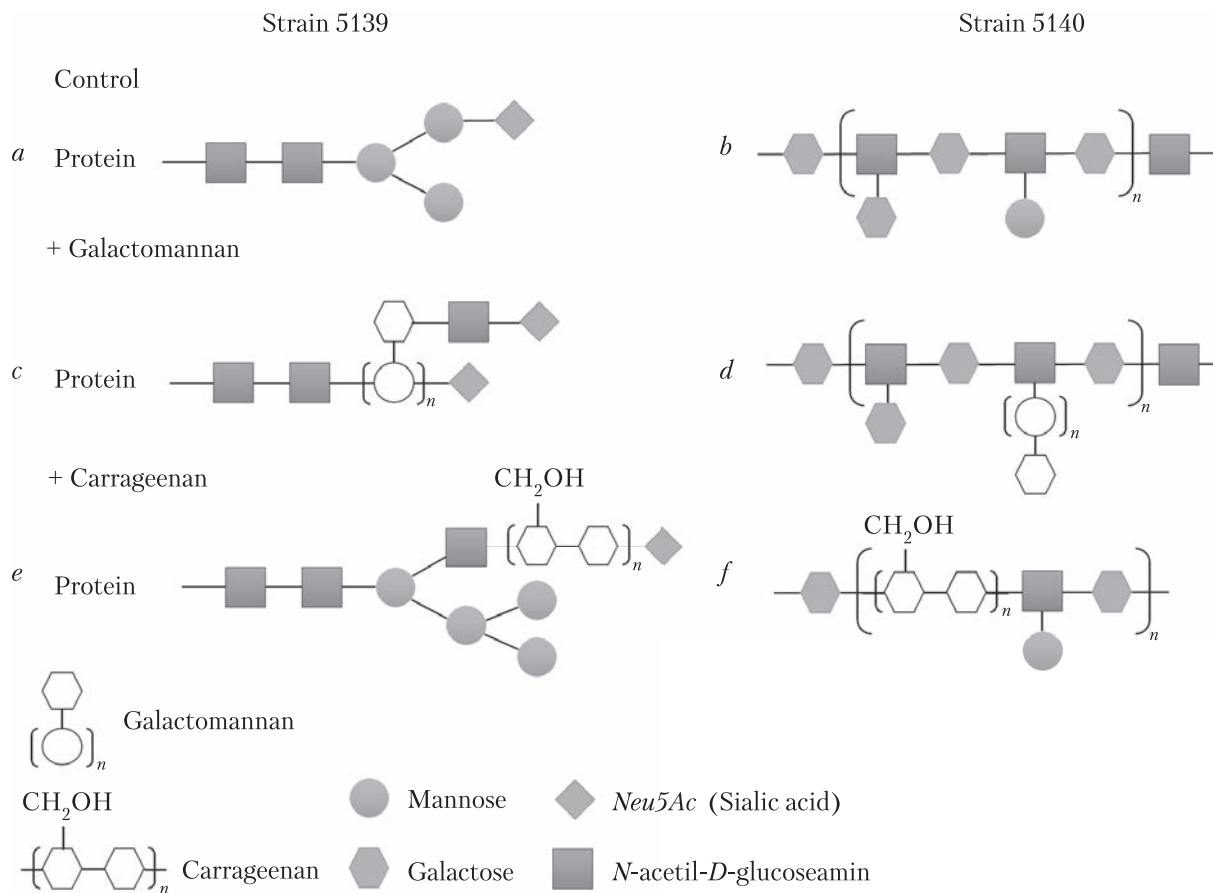
**Fig. 2.** Lectin-gold particles binding with sugar residues within the extracellular matrix of in the presence of galactomannan *B. amyloliquefaciens* subsp. *plantarum* strains UCM B-5139, 5140 (10 µg/mL), carrageenan (10 µg/mL) and carrageenan nanocomposite (marked as “Carrageenan NC”) (10 µg/mL). The mark “n/a” determines the samples in which lectins were not added. “PHA” is a lectin from *Phaseolus vulgaris* (red kidney bean) highly specific to galactose, *N*-acetyl-*D*-glucosamine and mannose, “WGA” is a wheat germ agglutinin with a high specificity to *N*-acetyl-*D*-glucosamine and sialic acid, “LCA” is a *Lens culinaris* agglutinin highly specific to *D*-mannose and *D*-glucose

cosamine; however, this specificity is much lower than the binding capacity of chitin-binding lectin WGA. The WGA preferentially binds to dimers and trimers of *N*-acetyl-*D*-glucosamine and to sialic acid in the case of its terminal position [10]. The PHA specifically binds to mannose and galactose residues and to *N*-acetyl-*D*-glucosamine in case of their inner position [8].

The cells of both bacterial strains, used in the study, showed a high affinity to lectin PHA, however, the amount of PHA, as well as others lectins, was 4 times higher in the extracellular matrix of cells of strain 5140 (Fig. 2). The WGA showed almost twice lower binding activity to the extracellular matrix of cells of both strains, while LCA had comparable to WGA binding to the components of the extracellular matrix of cells of strain 5139 and twice lower capacity for the cells of strain 5140. Such a result indicates that extracellular matrices surrounding the cells of these two strains are different. Therefore we evaluated the quantitative differences between binding capacities of lectins to various sugars to understand the potential structure of the polysaccharides within the extracellular matrix.

We determined the number of the specific sugar residues within extracellular matrices of bacteria and found that mannose and glucose were the major sugars in the matrix of the cells of strain 5139, while galactose was the dominant sugar in the matrix of cells of strain 5140 (Table 1). In view of the content and the ratio of each sugar residue, we could assume that the general composition of the extracellular polysaccharide complex of cells of strain 5139 is  $Man_3GluNAc_2Neu5Ac$

that means that, on average, there are two molecules of *N*-acetyl-*D*-glucosamine and one molecule of sialic acid per every 3 molecules of mannose. This structure is similar to the branched structure of *N*-glucans [11] (Fig. 3). The cells of strain 5140 contain an excess of *D*-galactose, an increased quantity of *N*-acetyl-*D*-glucosamine, while the presence of sialic acid is doubtful, and therefore



**Fig. 3.** Hypothetical structure of the extracellular exopolysaccharides of *B. amyloliquefaciens* subsp. *plantarum* strains UCM B-5139, 5140

**Table 1. The sugar residues determined within extracellular matrix of bacteria cells according to the lectins affinity**

Sugar residue	Features	Lectin binding capacity (in %) with ECM	
		Strain 5139	Strain 5140
<i>D</i> -mannose ( <i>Man</i> ), <i>D</i> -glucose ( <i>Glu</i> )	Branched	27.7	15.2
<i>D</i> -galactose ( <i>Gal</i> )	Linear	0	34.9
<i>N</i> -acetyl- <i>D</i> -glucosamine ( <i>GluNAc</i> )	Dimers and/or oligomers	16.9	24.9
Sialic acid ( <i>Neu5Ac</i> )	At the terminal ends	10.8	0
Hypothetical exopolysaccharide complex		$Man_3GluNAc_2Neu5Ac$	$Gal_3Man_1GluNAc_2$

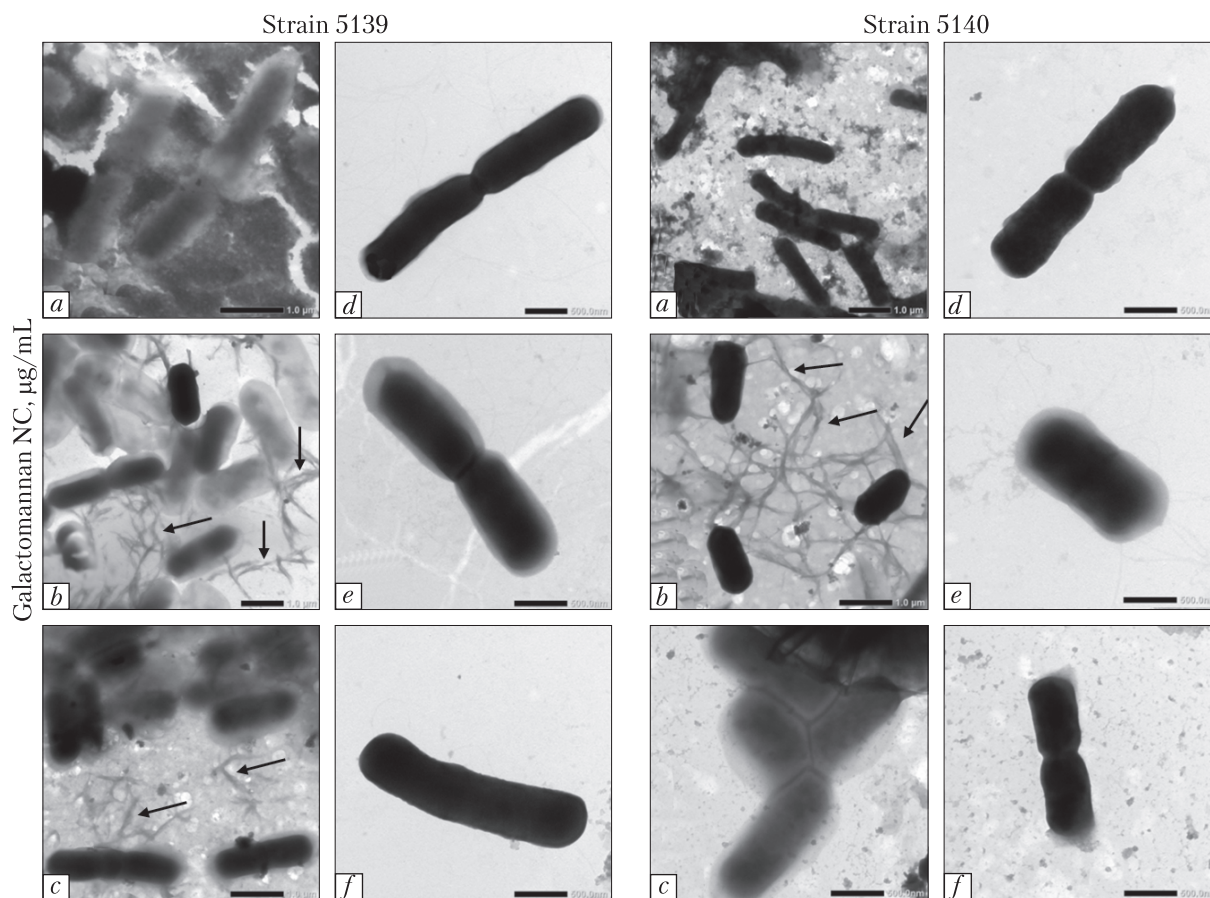
the general composition of their extracellular polysaccharides is  $Gal_3Man_1GluNAc_2$ . This composition suggests that the extracellular polysaccharides of cells of this strain may have a linear structure [11] (Fig. 3).

Galactomannan and carrageenan that we used in the study both are polysaccharides. The first one is composed of a mannose backbone with a side-linked galactose and the second one is a linear sequence of galactose and 3,6-anhydrogalactose. In the presence of galactomannan and carrageenan, significant changes in both the quantitative and qualitative characteristics of lectins-binding to the extracellular matrix were noted (Fig. 2). Galactomannan increased the quantity of *N*-acetyl-*D*-glucosamine and sialic acid residues in the extracellular matrix of cells of strain 5139, and had no effect on *D*-mannose/*D*-glucose content. While in the strain 5140, galactomannan did not cause significant changes in comparison with control. Carrageenan increased the quantity of *N*-acetyl-*D*-glucosamine and *D*-mannose/*D*-glucose in the matrix of cells of strain 5139 and reduced the quantity of *N*-acetyl-*D*-glucosamine in the extracellular matrix of cells of another strain.

This result indicated a strain-specific influence of galactomannan and carrageenan on bacterial extracellular matrix composition. We suggested that galactomannan is able to incorporate into the synthesizing polysaccharides instead of mannose. In this case, it can be a mediator for the creation of new polysaccharides. As a result, we get an increased amount of *GluNAc* and *SiAd*, but not mannose. This assumption is in agreement with the marked changes in the quantities of sugar residues in the extracellular matrix of cells of strain 5139. In the case of the cells of another strain (5140), galactomannan did not change the polysaccharide structure, because mannose was not its main element, but linked as a side-group. Thus, galactomannan is seemed to be able to binds to an existing polysaccharide as a side-group too (Fig. 3).

The influence of carrageenan nanocomposite (CG-Nano) causes no quantitative differences in comparison to the pure carrageenan action in the case of cells of strain 5139. However, more lectins were concentrated closer to the cell wall surface, and this can suggest that shorter polysaccharide chains were created, or that they have stick to each other. In the case of cells of strain 5140, an increase of binding of LCA and WGA was marked, indicating that quantities of *N*-acetyl-*D*-glucosamine and *D*-mannose/*D*-glucose residues increased in the extracellular matrix of these cells. Nevertheless, the simultaneous decrease of the binding capacity of PHA indicated that the number of galactose residues, as well as *N*-acetyl-*D*-glucosamines that had inner positions, were both reduced. This may be a result of the complete change of the structure of exopolysaccharides and formation of a set of new polysaccharides with *N*-acetyl-*D*-glucosamine and *D*-mannose/*D*-glucose.

The galactomannan nanocomposite (GM-Nano) suppressed lectin-binding activity for these strains of bacteria, and, therefore, it was studied at lower concentrations as well (1, 5, and 10  $\mu\text{g/mL}$ ). The suppression was dose-dependent with the maximum at 10  $\mu\text{g/mL}$  of the nanocomposite. Cells of the strain 5139 were more sensitive to the presence of this nanocomposites, and lectin-binding activity was almost completely suppressed at a concentration of 1  $\mu\text{g/mL}$ . The lectin-binding capacity of the exopolymeric matrix of cells of strain 5140 increased 1.5-2.5 times at 1  $\mu\text{g/mL}$  of this compound and dose-dependently decreased at 5 and 10  $\mu\text{g/mL}$  (Table 2). The high affinity of the cells of strain 5139 to WGA, specific to *GluNAc* and sialic acid, was marked at a concentration of nanocomposite 10  $\mu\text{g/mL}$  ( $124.7 \pm 10.3 \text{ un}/\mu\text{m}^2$  in comparison to  $41.1 \pm 10.2 \text{ un}/\mu\text{m}^2$  in control).



**Fig. 4.** Ruthenium red binding with the extracellular matrix of *B. amyloliquefaciens* subsp. *plantarum* strains UCM B-5139, 5140 in the presence of galactomannan nanocomposite. The black arrows on subfigures *b* and *c* indicate the filament-like structures

**Table 2. Lectin-binding activity (units per  $\mu\text{m}^2$ ) of *B. amyloliquefaciens* subsp. *plantarum* strains UCM B-5139, 5140 treated with galactomannan nanocomposite. Mean  $\pm$  Sd**

Lectin and its specificity	Galactomannan nanocomposite, $\mu\text{g/mL}$		
	0 (control)	1.0	5.0
Strain 5139			
PHA: <i>D</i> -galactose, <i>D</i> -mannose, <i>N</i> -acetyl- <i>D</i> -glucosamine	66.3 $\pm$ 7.5	10.2 $\pm$ 3.1	14.8 $\pm$ 4.2
WGA: <i>N</i> -acetyl- <i>D</i> -glucosamine, sialic acid	41.1 $\pm$ 10.2	16.0 $\pm$ 4.5	3.9 $\pm$ 2.1
LCA: <i>D</i> -mannose and <i>D</i> -glucose	40.5 $\pm$ 7.1	4.0 $\pm$ 3.1	3.0 $\pm$ 2.9
Sum of lectins	147.9 $\pm$ 24.8	30.2 $\pm$ 10.7	21.7 $\pm$ 9.2
Strain 5140			
PHA: <i>D</i> -galactose, <i>D</i> -mannose, <i>N</i> -acetyl- <i>D</i> -glucosamine	240.7 $\pm$ 22.0	320.5 $\pm$ 19.7	0
WGA: <i>N</i> -acetyl- <i>D</i> -glucosamine, sialic acid	100.1 $\pm$ 8.7	237.4 $\pm$ 22.1	74.9 $\pm$ 9.3
LCA: <i>D</i> -mannose and <i>D</i> -glucose	61.3 $\pm$ 13.3	72.2 $\pm$ 10.2	54.5 $\pm$ 11.1
Sum of lectins	402.1 $\pm$ 44.0	630.1 $\pm$ 52.0	129.4 $\pm$ 20.4



There are at least two possible explanations of the marked effects of these nanocomposites. The first one is the competitive binding of these nanocomposites with bacterial exopolysaccharides, and, the second one, is a blockade of the synthesis of exopolysaccharides in the presence of the nanocomposites. To check if there were any signs of the blockade, we, first of all, visualized the extracellular matrix with ruthenium red that has specificity to mucopolysaccharides (the long unbranched sequences of repeating disaccharide units, which consist of an amino sugar (*N*-acetyl-*D*-glucosamine or *N*-acetyl-*D*-galactosamine) along with a uronic sugar or galactose). It was shown that bacterial cells of both strains were submerged into a quite homogeneous matrix (Fig. 3, *a-c*) to which ruthenium molecules were well attached indicating that both of them contain acidic mucopolysaccharides [12]. The free forms of bacteria were not stained with ruthenium red (Fig. 4, *d-f*), but were able to bind with WGA (Fig. 2). This suggests that the extracellular matrix of the free forms of bacteria cells contains no acidic mucopolysaccharides, but still contains *GluNAc* moieties. The galactomannan nanocomposite (GM-Nano) caused no significant influence on the electron density of the extracellular matrix of bacteria of strain 5140 and reduced a density of the matrix around the cells of strain 5139 (Fig. 4, *b, c*). The reason for such differences can be in the changes of the structure of the matrix (from the granule-like in the control to the homogeneous one) that take place in the presence of this nanocomposite, rather than in the suppression of the synthesis of exopolymers. In addition, the galactomannan nanocomposite caused a formation of the short (1-2  $\mu\text{m}$ ) fibers of unclear nature, which were best noticed at 5  $\mu\text{g}/\text{mL}$  of the nanocomposite (Fig. 4, *b*). Therefore, we can assume that nanocomposites did not cause a blockade of the synthetic processes of extracellular polymers, and rather structural changes of the existing exopolysaccharides took place in the presence of this nanocomposite.

**Conclusion.** The exopolysaccharide matrix of bacteria and fungi is pivotal in the numerical processes associated with intercellular interaction in symbiotic and parasitic systems [13], adaptation to living conditions [14], adhesion to biotic and abiotic substrates, formation of biofilms [15], etc. In the current study, we determined that, in addition to the direct action of polysaccharides and their nanocomposites on the properties of the mammalian epithelial cells or gut microflora, with which they will contact as the prebiotic substances, these compounds may cause an indirect impact through the changes of properties of the bacterial extracellular matrix, in case of their use as the components of synbiotic preparations. Both compounds, carrageenan and galactomannan, and their nanocomposites showed a potency to change the content of the extracellular matrix of bacilli probiotic cells. Their effect depended, first of all, on the structural properties of the bacterial polysaccharides, since it seems obvious that these compounds (being the polysaccharides themselves) were, presumably, able to become a part of the corresponding bacterial polysaccharide chains. The nanocomposites of these compounds caused less predictable structural changes of the extracellular polysaccharides, and high concentrations were able totally to prevent lectins-binding. However, the synthesis of extracellular polysaccharides by *Bacillus* strains was not blocked in the presence of these nanocomposites. Considering the results of this study, it should be reasonable to check the ability of these compounds to influence various biological peculiarities of the treated cells. These questions are not touched here, for example, their motility, adhesive properties, etc., since these, along with other cell properties, are the factors of virulence that directly depend on the structure of exopolysaccharides.

## REFERENCES

1. Markowiak, P. & Śliżewska, K. (2017), Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, 9, 1021. <https://doi.org/10.3390/nu9091021>
2. Rostami, F. M., Mousavi, H., Mousavi, M. R. N. & Shahsafi, M. (2018). Efficacy of probiotics in prevention and treatment of infectious diseases. *Clin. Microbiol. Newsletter*, 40, No. 12, pp. 97-103. <https://doi.org/10.1016/j.clinmicnews.2018.06.001>
3. Guarner, F., Sanders, M. E., Eliakim, R., Fedorak, R., Gangl, A., Garisch, J., Kaufmann, P., Karakan, T., Khan, A. G., Kim, N., De Paula, J. A., Ramakrishna, B., Shanahan, F., Szajewska, H., Thomson, A. & Le Mair, A. (2017). WGO Practice Guideline — Probiotics and prebiotics. Milwaukee: WGO.
4. Elshaghabee, F. M. F., Rokana, N., Gulhane, R. D., Sharma, C. & Panwar, H. (2017). *Bacillus* as potential probiotics: status, concerns, and future perspectives. *Front Microbiol.*, 8, 1490. <https://doi.org/10.3389/fmicb.2017.01490>
5. Safronova, L. A., Zelena, L. B., Klochko, V. V. & Reva, O. N. (2012). Does the applicability of *Bacillus* strains in probiotics rely upon their taxonomy? *Can. J. Microbiol.*, 58, No. 10, pp. 212-219. <https://doi.org/10.1139/w11-113>
6. Safronova, L. A., Didenko, G. V., Podgorsky, V. S., Sukhov, B. G. & Dzhoiev, Yu. P. (2014). Immunomodulatory activity of new galactose-containing polysaccharides. *Lik. Sprava*, No. 9-10, pp. 64-70 (in Russian).
7. Lesnichaya, M. V., Sukhov, B. G., Sapozhnikov, A. N., Safronova, L. A., Evseenko, O. V., Ilyash, V. M., Podgorskii, V. S. & Trofimov, B. A. (2014). New nanobiocomposites of ammonium magnesium phosphate and carrageenan as efficient prebiotics. *Dokl. Chem.*, 457, Pt. 2, pp. 144–147. <https://doi.org/10.1134/S0012500814080023>
8. Stoitsova, S., Ivanova, R. & Dimova, I. (2004). Lectin-binding epitopes at the surface of *Escherichia coli* K-12: examination by electron microscopy, with special reference to the presence of a olonic acid-like polymer. *J. Basic Microbiol.*, 44, No. 4, pp. 296-304. <https://doi.org/10.1002/jobm.200410350>
9. Stanley, P., Schachter, H. & Taniguchi, N. (2009). N-Glycans. In Varki A., Cummings R.D., Esko J.D. et al. (Eds.). *Essentials of Glycobiology*. Chapter 8. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press. Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK1917/>
10. Itakura, Y., Nakamura-Tsuruta, S., Kominami, J., Tateno, H. & Hirabayashi, J. (2017). Sugar-binding profiles of chitin-binding lectins from the hevein family: a comprehensive study. *Int. J. Mol. Sci.*, 18, No. 6, 1160. <https://doi.org/10.3390/ijms18061160>
11. Sakurai, M. H., Kiyohara, H., Nakahara, Y., Okamoto, K. & Yamada, H. (2002). Galactose-containing polysaccharides from *Dictyostelium mucoroides* as possible acceptor molecules for cell-type specific galactosyl transferase. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, 132, No. 3, pp. 541-549. [https://doi.org/10.1016/s1096-4959\(02\)00067-2](https://doi.org/10.1016/s1096-4959(02)00067-2)
12. Luft, J. H. (1971). Ruthenium red and violet. II. Fine structural localization in animal tissues. *Anat. Rec.*, 171, pp. 369-416. <https://doi.org/10.1002/ar.1091710303>
13. Colvin, K. M., Gordon, V. D., Murakami, K., Borlee, B. R., Wozniak, D. J., Wong, G. C. & Parsek, M. R. (2011). The pel polysaccharide can serve a structural and protective role in the biofilm matrix of *Pseudomonas aeruginosa*. *PLoS Pathog.*, 7, No. 1, e1001264. <https://doi.org/10.1371/journal.ppat.1001264>
14. Ophir, T. & Gutnick, D. L. (1994). A role for exopolysaccharides in the protection of microorganisms from desiccation. *Appl. Environ. Microbiol.*, 60, pp. 740-745.
15. Vu, B., Chen, M., Crawford, R. J. & Ivanova, E. P. (2009). Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules*, No. 14, pp. 2535-2554. <https://doi.org/10.3390/molecules14072535>

Received 18.05.2020

Л.А. Сафронова, С.І. Войчук,  
О.С. Броварська

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, Київ  
E-mail: safronova\_larisa@ukr.net

ВПЛИВ НАНОБІОКОМПОЗИТІВ  
НА ЕКЗОПОЛІСАХАРИДНИЙ МАТРИКС  
ШТАМІВ РОДУ *BACILLUS*

Вивчено вплив нанобіокомпозитів карагінану та галактоманану — перспективних пребіотиків — на формування позаклітинних полісахаридних комплексів пробіотичних штамів *Bacillus amyloliquefaciens* subsp. *plantarum* UCM В-5139 та UCM В-5140. Аналіз даних показав, що бактеріальні штами мали різницю у вмісті різних залишків цукру в їх екзополісахаридних матрицях. Карагінан, галактоманан і їх нанобіокомпозити характеризувалися здатністю до зміни вмісту позаклітинного полісахаридного матриксу пробіотичних клітин бацил. Вплив цих сполук залежав, імовірно, від природних структурних властивостей полісахаридів досліджених штамів. Синтез екзополісахаридів пробіотичними штамми не був заблокований у присутності досліджуваних нанобіокомпозитів. Отримані результати свідчать про можливу подвійну (пряму та непряму) дію полісахаридів і їх нанобіокомпозитів у разі їх використання як пребіотичних компонентів синбіотичних препаратів: шляхом прямої біологічної дії на властивості клітин епітелію ссавців або мікрофлори кишечника та опосередковано через зміни властивостей позаклітинної полісахаридної матриці пробіотичних штамів.

**Ключові слова:** пробіотики, штами роду *Bacillus*, пребіотики, нанобіокомпозити, полісахаридний матрикс.