UDK 577.1+579.8

### V.S. Pidgorskyi, E.O. Kovalenko, O.V. Sashchuk, K.I. Getman

Zabolotny Institute of Microbiology and Virology, National Academy of Science of Ukraine, 154 Acad. Zabolotny St., Kyiv, 03143, Ukraine

# **BACILLI LECTINS AND THEIR TARGETS**

It were revealed structural features of extracellular sialic acid-spesific lectin from saprophytic strain of Bacillus subtilis IMV B-7014 and shown this lectin constituted the complex of molecular forms (isoforms, isolectins) that obtained the working names BSL1, BSL2 and BSL3 with mM 50 kDa, 40kDa and 55 kDa respectively. They differed in physical, chemical and biological characteristics. It were investigated direct and indirect impact mechanisms of the lectin and their molecular forms. We explored the lectin showed the greatest affinity to sialic acid-containing glycoconjugates where terminal O-acetylated sialic acid associated with subterminal D-galactose via  $\alpha 2,3$ -,  $\alpha 2,6$ - or  $\alpha 2,8$ -links. Bacilli lectins completely blocked surface sialic acid-containing receptors of influenza, herpes, hepatitis C viruses and HIV and thus prevented not only their adsorption and reproduction, but also appearance and further development of viral infection. It were shown multidirectional action of the lectin isoforms on proliferation of mammalian cell cultures with different origins: normal cells (primary mouse fibroblasts), relatively normal cells (epithelioid cells, Chinese hamster ovary) and cancer cell line of HeLa. We identified an influence of gene functional condition of reparation system of bacilli cells on ability to lectin synthesis by bacteria. Using the model system RNA-polymerase of bacteriophage T7 it were established the isolectins had different effects on the yield of RNA-transcription product in vitro. K e y w o r d s: Bacillus subtilis, lectins, isolectins, isoforms, sialic acid, antivirus

activity, reparation, transcription, recP.

Introduction. Lectins are a heterogeneous group of proteins capable of binding carbohydrates selectively and reversibly without inducing changes in the chemical structure of the latter. These proteins were found in all living systems from viruses to humans and play a fundamental role in carbohydrateprotein recognition [6, 31, 33, 34]. Lectins were first detected in plants, and the commercial production of lectins from plant sources is considered the most costeffective approach [33, 34]. Lectins of higher animals and humans have recently become the object of intensive investigation, and their involvement in biological processes of crucial importance, such as fertilization, embryogenesis, immune response, cell growth, proliferation, and others, has been demonstrated [11, 12, 33, 34]. A considerable body of data on lectins from pathogenic organisms and their role in the initiation and development of pathological process has been collected [6, 33, 34], while lectins from saprophytic microorganisms have been out of the scope of researchers' interest for a long time, the particular features and biological role of these proteins remaining virtually uncharacterized [6, 11, 13, 33, 34]. These lectins are supposedly involved in such physiological processes as bacterial adhesion, communication, formation of bacterial films and aggregates, growth and division, adaptation, protection from stress factors,

<sup>©</sup> V.S. Pidgorskyi, E.O. Kovalenko, O.V. Sashchuk, K.I. Getman, 2017

nutrient supply, toxin neutralization, and other functions of normal microflora [6, 11, 12, 13, 31, 33, 34]. The molecular mechanisms of lectin action that determine these physiological processes are still incompletely characterized.

It is known that the mechanisms of lectin action can be direct and indirect [6, 31, 33]. Direct mechanisms are based on specific binding of glycoconjugates on different cell surfaces by lectins. Typical examples are agglutination, precipitation and adhesion. Indirect effects are happening with involving cell receptors and complex signaling pathways, an examples of which are mitogenic stimulation, apoptosis and transcription [6, 31, 33].

Lectins are found in all living cells, but their qualitative and quantitative composition can vary considerably. A large-scale search conducted by the authors resulted in the detection of a large number of lectin- producing strains of various saprophytic microorganisms (bacilli, yeasts, lactic acid bacteria, corynebacteria and nocardia-like bacteria, actinomycetes, streptomycetes, and others) [26, 31, 32].

**Results.** Bacteria of the genus *Bacillus* were of particular interest since they can secrete large amounts of lectins into the cultivation medium, and this facilitates the process of lectin isolation [21]. Sources of extracellular lectins was saprophytic strains of *Bacillus subtilis* IMV B-7014 from Ukrainian Collection of Microorganisms of Zabolotny Institute of Microbiology and Virology (IMV), National Academy of Sciences, Ukraine, isolated from intestines of healthy newborn calf.

Hemagglutinative (lectin) activity (HA, LA) and carbohydrate specificity are the most important characteristics of lectins. One of the basic characteristics of lectins of saprophytic bacilli strains is their affinity to sialic acid. Bacilli lectins were able to differentiate certain sialic acid-containing structures. Their affinity for these structures decreased in the following order: NeuGc  $\rightarrow$  $\alpha$ -isomer of O-acetylated form of Neu5Ac  $\rightarrow$  Neu5Ac $\alpha$ 2-6  $\rightarrow$  Neu5Ac $\alpha$ 2-3  $\rightarrow$ Neu5Ac $\alpha$ 2-8  $\rightarrow$  acid residues [22].

Previously, we found a high antiviral activity of bacilli lectins relatively influenza, herpes and hepatitis C viruses and HIV and direct mechanism of antiviral effects. The study of antiviral activity of bacilli lectins against these viruses were carried out using one of the most active lectins – extracellular lectin of saprophytic strain of *B. subtilis* IMV B-7014 with affinity to sialic acid. Compared with other known antiviral drugs that are used at present, this lectin is the most active inhibitor of adsorption and reproduction of these viruses [22, 27–30].

As we have shown before, this extracellular lectins show the greatest affinity to sialic acid-containing glycoconjugates where terminal O-acetylated sialic acid associated with subterminal D-galactose via  $\alpha 2,3$ -,  $\alpha 2,6$ - or  $\alpha 2,8$ -links. The same structures with terminal glycoproteins are on the surface of influenza, herpes, hepatitis C viruses and HIV, which play a key role in target cells detection by viruses and initiation of infection. [27–30].

The mechanism of antiviral action of extracellular sialic acid-specific lectins of bacilli shown in Fig. 1.

According to the literature lectins mechanisms are not limited to direct protein-carbohydrate interactions. Ability to exhibit immunomodulating and interferon inducting activity are examples of mediated effects of bacilli lectins [21].

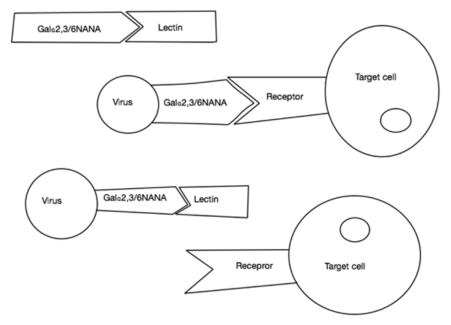


Fig. 1. Mechanisms of antiviral effects of bacilli extracellular sialic acid-specific lectins

Bacilli lectins exhibit distinct and differentiated immunomodulating effect on immunogenesis separate levels from changes in functional status of the bone marrow polypotent stem cells to activation of specific responses of T- and B-lymphocytes and their cooperative interactions in the immune response [21, 31]. Investigated lectins are inducers of natural gamma interferon synthesis and are compared with commercial preparations of plant lectins.

It is known that lectins exist as not individual molecules but a population of different molecular forms (isolectins) that presumably operate as a single regulator complex [6, 31, 33, 34]. According to this statement it were developed approaches to study of structural and functional heterogeneity of extracellular bacilli lectins, and an influence on mammalian cells proliferation *in vitro* as a basis for a possible use it as anticancer drugs [1, 9, 23–25].

Due to isoelectrofocusing method it were revealed differences between hemagglutinating substances in charge [9, 23, 24]. In accordance with it the substances concentrated in three pH zones: acidic, alkaline and intermediate. And they are assigned to working titles BSL1 (*B.subtilis* lectin), BSL2, and BSL3, respectively (Table).

Table

Characteristics of isolectins of strain Bacillus subtilis IMV B-7014

| Characteristic   |        | Isolectins          |                     |            |
|------------------|--------|---------------------|---------------------|------------|
|                  |        | BSL1                | BSL2                | BSL3       |
| pH zone          |        | 2.5-3.0             | 6.0-6.5             | 8.5-9.0    |
| HAA              | rabbit | 0                   | 16 HAU              | 1024 HAU   |
|                  | sheep  | 512 HAU             | 0                   | 0          |
| Carbohydrate     |        | Submandibular gland | N-acetyl-neuraminic | Sialactose |
| specificity      |        | mucin of bull       | acid                |            |
| Molecular weight |        | 50 kDa              | 40 kDa              | 55 kDa     |

In addition to the charge received isolectins differed in selectivity to erythrocytes with different species specificity, where BSL1 interacted only with sheep red blood cells, and others – with rabbit erythrocytes. Preparations were significantly different for hem agglutinating activity and arranged in descending order: BSL3> BSL1> BSL2. On the one hand, determination of carbohydrate specificity has allowed to identify a rare specificity to sialic acids in all the studied drug, and on the other hand, their individual capacity to recognize thin differences in structure of these acidic sugars.

The calculated molecular weight of isolectins that based on the ideas of their subunit structure (10 kDa and 25 kDa), have given the basis to assume that BSL1 is a homodimer with mM 50 kDa, BSL2 is homotetramer with mM 40 kDa, and BSL3 is heterotetramer MM about 55 kDa.

To study the isolectins effects on mammalian cells proliferation *in vitro* were used preparates BSL1 and BSL3 with an alternative charge, different fine specificity and higher lectin activity [9, 25]. The object of lectin impact were normal cells (primary mouse fibroblasts), relatively normal cells (epithelioid cells, Chinese hamster ovary) and cancer cell line of HeLa (Fig. 2).

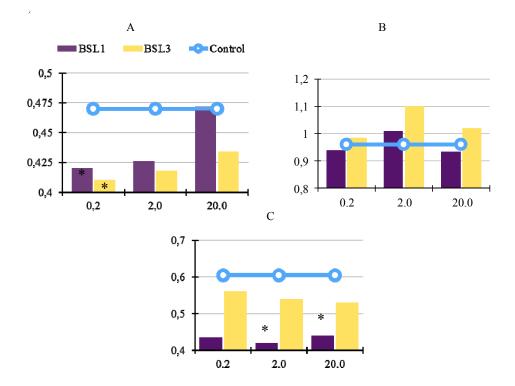


Fig. 2. Comparative effects of molecular forms of *B. subtilis* IMV B-7014 extracellular lectin on mammalian cells proliferation *in vitro*: primary mouse fibroblasts (A), epithelioid cells of Chinese hamster ovary (B), the cancer cell line HeLa (C). Isolectins: 1 – BSL1, 2 – BSL3, Control – control without lectin. x – concentration of preparation in mg / ml, y – an indicator of adsorption at 570 nm \* – significant deviation from control level (p <0,05)</li>

It were shown that *B. subtilis* isolectins differed in character effects on mammalian cells. Their effect was dependent on concentration and cell type. In culture of primary mouse fibroblasts both isolectins showed cytostatic ac-

tion that was reduced with increase of concentration (Fig. 2A). In contrast, cell lines of transplantable Chinese hamster were resistant to the cytostatic effect of these preparates. Moreover, isolectin BSL3 showed a tendency to stimulate the growth of the cell type, more pronounced at a concentration of 2 mg / ml (Fig. 2B). Manifest differences in isolectin action were displayed by treatment of malignant transformed human HeLa cell line. In this case BSL1 significantly reduced their proliferative activity on 25–30 % in all range of concentration, unlike other isolectin BSL3 that weak cytostatic effects on cells were not significant (Fig. 2C).

*Bacillus subtilis* genome was completely sequenced and contains more than 5,500 sequences that code proteins. However bacilli lectin genes are not yet installed. We identified an influence of gene functional condition of bacilli cells reparation system on bacteria lectin synthesis ability with using of mutants with defect reparation system [8, 9]. It were established that the bacilli lectin activity depends on the *recP* gene product which was referred to postreplication reparation and recombination (Fig. 3).

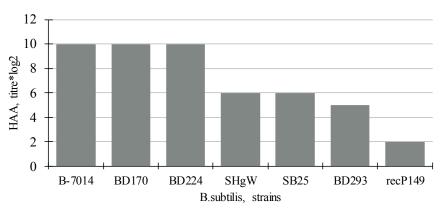


Fig. 3. Bacilli lectin activity dependence on recP gene product

We started to work on investigation of effects of saprophytic microorganisms lectins as broad spectrum regulatory proteins. To determine the ability of bacilli lectins influence on regulation of transcription we used model system RNA-polymerase of bacteriophage T7 *in vitro* [9]. That system applied for comparative study of regulatory properties of bacilli extracellular lectin isoforms.

In previous studies we obtained three isoforms of extracellular lectin from *B. subtilis* B-7014 which differed in physical, chemical and biological characteristics. These molecular forms had been investigated separately in inhibition of transcription *in vitro* with DNA-dependent RNA-polymerase from bacteriophage T7. Electrophoretic analysis of transcription products are presented in Fig. 4.

It were established that the lectin isoforms have different effects on the yield of RNA-transcription product in vitro. Isoform BSL3 (alkaline pH zone) completely blocks the transcription process from plasmid promoter (Fig. 4: A-3 and B-3). Isoform BSL1 (acidic pH zone) slightly activates (10 %) formation of transcripts (Fig. 4: A-1 and B-1) as compared to controls (Fig. 4: A-K and B-K). An intermediate form lectin BSL2 (neutral pH zone) does not effect significantly on the process (Fig. 4: A-2 and B-2).

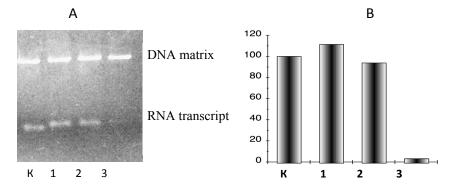


Fig. 4. Electrophoregramm (A) and densitogramm (B) of transcription product *in vitro*, that were obtained under the influence of extracellular *B. subtilis* IMV B-7014 molecular forms. K (checking) – full transcript obtained in the absence of lectin; lectin isoforms: 1 - BSL1, 2 - BSL2, 3 - BSL3

The results indicate functional differences of the lectin isoforms, one of which blocks the transcription process. This multiplicity of structural molecular forms of the extracellular lectin from saprophytic microorganism *B. subtilis* IMV B-7014 are the basis of multidirectional regulatory lectin action on the most important intracellular processes, including the transcription process.

To sum up we have established the regulatory action of lectin isoforms on transcription and its dependence on the structural features of the molecule. Lectin activity of bacilli depends on *recP* gene product, which refers to the postreplicative reparation and recombination systems. One target of this lectin can be DNA-dependent RNA synthesis.

**Discussion.** It were shown that many human pathogens utilize cell surface glycans as either receptors or ligands to initiate adhesion and infection [33]. Although the real role of extracellular bacilli lectins in these bacteria has not been yet established, their ability to discriminate structural details and linkage of animal and human sialic acids and sialic glycoconjugates can demonstrate the functional significance or importance of such linkages for the basic processes of biological recognition [22].

*Escherichia coli*, for example, binds to host mannosides, while influenza virus binds to host sialic acids [18]. Other strains of *E. coli* have been discovered that demonstrate specificities toward other host cell surface carbohydrate moieties such as galabiose (Gal- $\alpha$ -4-Gal) and NeuAc- $\alpha$ 2,3-Gal- $\beta$ 3-GalAc [2, 10]. The genital pathogen *Neisseria gonorrhea* specifically binds N-acetyllactosamine (Gal- $\beta$ -4-GlcNAc), and Straptococcus pneumonia specifically binds the pentasaccharide NeuAc- $\alpha$ -3-Gal- $\beta$ -4-GlcNAc- $\beta$ -3-Gal- $\beta$ -4-Glc as well as internal tetra- and trisaccharides Gal- $\beta$ -4-GlcNAc- $\beta$ -3-Gal- $\beta$ -4-Glc and GlcNAc- $\beta$ -3-Gal- $\beta$ -4-Glc respectively. *Pseudomonas auruginosa* specifically binds fucose (L-Fuc). Bacteria can discriminate between two identical glycans that differ in only one hydroxyl group [33]. Such host-pathogen interactions are multivalent, and therefore the binding events are of high affinity and suited for host invasion [18].

We showed a high antiviral activity of *B.subtilis* IMV B-7014 lectin relatively influenza, herpes, hepatitis C viruses and HIV. Bacilli lectin completely blocks surface sialic acid-containing receptors (Gal- $\alpha$ 2,3/6NANA) of these vi-

ruses and thus prevents not only their adsorption and reproduction, but also appearance and further development of viral infection [27–30]. The most popular research aspects in antiviral activity of lectins are connected with HIV. A lectin (D-mannose-spesific) from *Gerardia savaglia* was for the first time reported to prevent infection of H9 cells with HIV-1. Furthermore, the lectin inhibited syncytium formation in the HTLV-IIIB/H9-Jurkat cell system and HIV-1/human lymphocyte system by reacting with the oligosaccharide site chains of the HIV-1 gp120 envelop molecule (high-mannose oligosaccharides) [19]. Banana (*Musa acuminata*) lectin directly bound the HIV-1 envelope protein gp120 and blocked entry of the virus into the cell, and decreased the levels of the strongstop product of early reverse transcription [36]. The treatment of AIDS with lectins is being investigated in many studies. Different lectins have different anti-HIV mechanisms.

As lectin from other sources bacilli lectins present a complex of isoforms with different biological, chemical and physical properties. Lectin from the «old» seeds (more than one year since harvested) of Bandeiraea (Griffonia) simplicifolia contains three isolectins GS I, GS II and GS IV. GS I mainly shows anti-B activity (A, B, O and AB-blood group) but it also shows some activity against A, N and Tn antigens. GS II is considered to react with Leb and Y antigens [35]. Later on, Lescar et al studied the crystal structure of GS I. They found that this isolectin contains two different subunits A and B, which combine to form five different tetrameric structures made up different proportions of two subunits. They are A4, A3B, A2B2, AB3 and B4 with different binding specificities [14, 20]. The A subunit is specific for  $\alpha$ GalNAc but it also recognizes αGal. So it agglutinates both A and B blood group RBCs. The B subunit is specific for only  $\alpha$ Gal end group and so it agglutinates only B group RBCs. Therefore, GS I B4 is used for the detection of αGal residues in biological material. e.g., tissue from human breast carcinomas [7]. The GS I A4 has a strong affinity for the Forssman antigen (αGalNAc 1-3 GalNAc) and the Tn (αGalNAc- Ser/Thn) antigen [37].

By Flory L.L. it has been observed that two different types of lectins are present in *Ulex europeaus* seed extract [5]. They are named Ulex I and Ulex II. Ulex I is inhibited by L-fucose but Ulex II is not inhibited by L-fucose and it is inhibited by di-N-acetylchitobiose, a sugar with an N-acetylglucosaminyl residue. It likely that Ulex II reacts with subterminal N-acetylglucosamonyl residue in the H structure (H antigen from A, B, O and AB blood group) but only in the presence of terminal L-fucose [17].

Our investigation of bacilli lectins showed that they were similar to other lectins (lectins were isolated from animal, plants, etc.) for the principles of the structure organization (subunits, isoforms) and biological properties.

Owing to their fine specificity, lectins have various applications in biomedical sciences including cancer research. It is well documented that lectins have an antitumor effect. Plant lectins represent a well-defined and a novel non-traditional source of anticancer compounds. Legume lectins are one of the most extensively studied plant lectin families for their molecular basis of the protein-carbohydrate interactions for several decades [4]. In recent years, the main interests in this lectin family lay in their potential application as antitumor agents that could bind specific cancer cell surface glycoconjugates.

Conconavalin A (ConA), a typical legume lectin with a mannose/glucose-

binding specificity, was reported to induce apoptosis in murine macrophage PU5-1.8 cells through clustering of mitochondria and release of cytochrome c. Recent study has showed that ConA induces apoptosis in human melanoma A375 cells in a caspase-dependent pathway. Subsequently, ConA caused mitochondrial transmembrane potential collapse, cytochrome c release, activation of caspases and eventually triggering a mitochondria-mediated apoptosis [15]. Another typical legume lectin with specificity towards sialic acid purified from *Phaseolus coccineus* L. (*Phaseolus multiflorus* wild) seeds possesses a remarkable anti-prolifirative activity. This lectin induced the caspase dependent apoptosis in murine fibrosarcoma L929 cells [16]. Besides, its antineoplastic activity was decreased abruptly when the sialic acid-specific activity was completely inhibited, which indicates that this sugar-binding specificity migh be the main reason sparking off the antineoplastic activity and apoptosis [3].

The study of *B. subtilis* IMV B-7014 isolectins and their impact on mammalian cell prolifiration in vitro showed their multidirectional effects so as the stimulation of growth of cells and their suppression. Ambiguous results of research in this area is probably explained by the fact that the different lectins, as well as their structural variants can interact with different surface receptors and activate different signaling systems. If we consider the lectins as perspective anticancer drugs, it is necessary to take into account their structural and functional heterogeneity. Only any one isoform may have cytostatic effect, while others may stimulate cell division or no effect on this process. This assumption evidenced by our results on the effect of *B. subtilis* isolectins on mammalian cells proliferation in culture.

Generally speaking, the above-mentioned discoveries of the lectins suggest that they migth possess more similar biological activities and anti-tumor mechanisms that are closely correlated with their corresponding molecular structures.

Within the past years lectins have become a well-established means for understanding varied aspects of cellular activity. Evidence is now emerging that lectins are dynamic contributors to tumor cell recognition (surface markers), cell adhesion and localization, signal transduction across membranes, mitogenic stimulation, augmintation of host immune defence, cytotoxicity and apoptosis. Despite this immediate mechanisms of lectin impact on intracellular processes is still poorly understood. Our researches of the properties and characteristics of saprophytic bacteria lectins affect only certain aspects of their influence on intracellular processes and vital functions of cells and the organism as a whole and thus have broad perspectives for its continuation in the future.

**Conclusions.** Saprophytic bacilli lectins have a wide range of pharmacological properties, namely antiviral, antitumor and immunomodulating activities, which cause a great interest.

As a result of studies the structural features of extracellular sialic acidspecific lectin from saprophytic strain *Bacillus subtilis* IMV B-7014 were determined and it was shown that this lectin presents a complex of molecular forms with molecular weights of 50 kDa, 40 kDa and 55 kDa, which differ in their physical, chemical and biological characteristics. Regulatory action of the lectin isoforms on the transcription process *in vitro* and its dependence on the structural characteristics of lectin molecules were established. The antiviral effect of bacilli lectin implies a direct blocking of surface sialic acid-specific receptors of influenza, herpes, hepatitis C viruses and HIV and inhibition of virus surface receptors adhesion to target cells. Thus, it prevents further viral adsorption and reproduction, as well as emergence and development of disease.

The indirect mechanism of lectin action is associated with cell membrane receptors and leads to launch of multiple intracellular signaling pathways. This mechanism of action is prevalent in humans and animals, is mediated by cell membrane receptors and leads to the development of immunomodulatory effects.

Multidirectional action of lectin isoforms on the proliferation is shown in mammalian cell cultures of different origins: normal cells (primary mouse fibroblasts), relatively normal cells (epithelioid cells, Chinese hamster ovary), and cancer cell line of HeLa. BSL1 and BSL3 isoforms showed cytostatic effect in relation to the normal cells, which decreased with the lectin concentration increasing. BSL3 isoform showed a stimulating effect on the epithelioid cells of Chinese hamster ovary, while BSL1 isoform did not affect the latter. Proliferation of the cancer cell line of HeLa decreased by 25–30 % over the entire range of concentrations after treatment by isolectin BSL1, while BSL3 impact was minor.

The lectin activity of bacilli depends on the *recP* gene product, which belongs to the postreplicational repairation and recombination.

Using the model system of bacteriophage T7 RNA-polymerase it was established that the DNA-dependent RNA synthesis is one of the targets of *Bacillus subtilis* IMV B-7014 lectin action.

**Perspectives.** Thus, the ability of saprophytic bacilli extracellular lectin to recognize the fine configuration of sialic acids molecules, and lectin isoforms impact on intracellular processes and development and growth of cell populations of different origins indicated their potential as the diagnostic and analytical reagents as well as pharmacological preparations.

# В.С. Підгорський, Е.О. Коваленко, О.В. Сащук, К.І. Гетьман

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, вул. Акад. Заболотного, 154, Київ, 03143, Україна

### ЛЕКТИНИ БАЦИЛ ТА ЇХ МІШЕНІ

#### Резюме

Виявлено структурні особливості позаклітинного сіалоспецифічного лектину сапрофітного штаму *Bacillus subtilis* IMV B-7014. Показано, що даний лектин представляє собою комплекс молекулярних форм (ізоформ, ізолектинів), які отримали робочі назви BSL1, BSL2 та BSL3 з mM 50 kDa, 40kDa і 55 kDa відповідно. Ізоформи різняться за фізичними, хімічними та біологічними характеристиками. Досліджено пряму та опосередковану дію лектину та його молекулярних форм. Показано, що найбільшу афінність лектин проявляє до сіаловмісних глікокон'югатів, де термінальна О-ацетильована сіалова кислота поєднана з субтермінальною D-галактозою зв'язками  $\alpha 2,3$ -,  $\alpha 2,6$ - або  $\alpha 2,8$ -. Даний лектин повністю блокує поверхневі сіаловмісні рецептори вірусів грипу, герпесу, гепатиту С та ВІЛ і, таким чином, запобігає не лише їх адсорбції та репродукції, а й виникненню і подальшому розвитку вірусної інфекції. Показана різнонаправлена дія ізоформ лектину на процеси проліферації у культурах клітин ссавців різного походження: нормальних клітин (первинні фібробласти миші), умовно нормальних клітин (епітеліоїдні клітини яєчнику китайського хом'яка) та трансформованих клітин лінії HeLa. Нами виявлено, що здатність бактерій до синтезу лектинів залежить від функціонального стану системи репарації бацилярних клітин. У системі PHK-полімерази бактеріофага T7 показано, що ізолектини по-різному впливали на вихід PHK-транскриптів *in vitro*.

*Ключові слова: Bacillus subtilis*, лектини, ізоформи, сіалова кислота, антивірусна активність, репарація, транскрипція, recP.

## В.С. Подгорский, Э.А. Коваленко, Е.В. Сащук, Е.И. Гетьман

Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, ул. Акад. Заболотного, 154, Киев, 03143, Украина

#### ЛЕКТИНЫ БАЦИЛЛ И ИХ МИШЕНИ

#### Резюме

Выявлены структурные особенности внеклеточного сиалоспецифического лектина сапрофитного штамма Bacillus subtilis IMV B-7014. Показано, что данный лектин представляет собой комплекс молекулярных форм (изоформ, изолектинов), которые были названы BSL1, BSL2 и BSL3 с молекулярной массой 50 kDa, 40kDa и 55 kDa соответственно. Изоформы отличались по физическим, химическим и биологическим показателям. Исследовано прямое и опосредованное действие лектина и его молекулярных форм. Показано, что наибольшую аффинность лектин проявляет по отношению к сиалосодержащим гликоконьюгатам, где терминальная О-ацетилированная сиаловая кислота соединена с субтерминальной D-галактозой связями α2,3-, α2,6- или α2,8-. Данный лектин полностью блокирует поверхностные сиалосодержащие рецепторы вирусов гриппа, герпеса, гепатита С и ВИЧ, и, таким образом, предотвращает не только их адсорбцию и репродукцию, но и возникновение и последующее развитие вирусного заболевания. Показано разнонаправленное действие изоформ лектинов на процессы пролиферации в культурах клеток млекопитающих разного происхождения: нормальных (первичные фибробласты мышей), условно нормальных (эпителиоидные клетки яичников китайского хомяка) и трансформированных клеток линии HeLa. Нами выявлено, что способность бактерий к синтезу лектинов зависит от функционального состояния системы репарации бациллярных клеток. В системе РНК-полимеразы бактериофага Т7 показано, что изолектины по-разному влияют на выход РНК-траскриптов in vitro.

Ключевые слова: Bacillus subtilis, лектины, изоформы, сиаловая кислота, антивирусная активность, репарация, транскрипция, recP.

- Brovarska O.S., Vasilyev V.M., Ostapchuk A.M., Kovalenko E.O., Sashchuk O.V. [Characteristics of extracellular lectin from saprophytic strain *Bacillus subtilis* IMV B-7014]. Microbiol Z. 2011; 73 (3): 33–38. Ukrainian.
- Buts L., Bouckaert J., De Genst E., Loris R., Oscarson S., Lahmann M., et al. The fimbrial adhesion F17-G of enterotoxigenic *Escherichia coli* has an immunoglobulinlike lectin domain that binds N-acetyl-glucosamine. Mol Microbiol. 2003; 49: 705–715.
- 3. *Chen J.B., Liu N., Ji. J., Zhou H.J., Bian C.Y., Li F., Chen S.J., et al.* A novel siakic acid-specific lectin from *Phaseolus coccineus* seeds with potent antineoplastic and antifungal activities. Phytomedicine. 2009; 16: 352–360.

- 4. *Damodaran D., Jeyakani J., Chauhan A., Kumar N., Chandra N.R., Surolia A.* Cancer lectin DB: a database of lectins relevant to cancer. Glycoconjugate J. 2008; 25: 191–198.
- Flory L.L. Differences in the H antigen on human buccal cells from secretor and nonsecretor individuals. Vox Sang. 1966; 11: 137–156.
- Gilboa-Garber N., Avichezer D., Garber N.C. Bacterial lectins: Properties, structure, effects, function and applications. In: Gabius H.J., Gabius S. (Eds.), Glycosciences: Status and Perspectives, Chapman and Hall. Weinheim, 1997: 369–398.
- Ito N., Imai S., Nagaike C., Morimura Y., Hatake K. Localization of binding sites of Ulex europaeus I, Helix pomatia I, Helix pomatia and Griffonia simplicifolia I-B4 lectins and analysis of their backbone structures by several glycosidasesand poly-Nacetyllactosamine-specific lectins in human breast carcinomas. Histochem Cell Biol. 1996; 106: 331–339.
- Karpova I.S., Kovalenko E.A., Koretskaya N.V., Getman E.I. [Extracellular lectins of saprophytyc bacteria *Bacillus subtilis* and its mutants] Biopolym Cell. 2004; 20: 290– 294. Russian.
- Karpova I.S., Kovalenko E.O., Sashchuk O.V., Getman K.I., Bilolipetska O.S. [Structural and functional polymorphism of *Bacillus subtilis* lectins]. XIII Congress of S.M. Vynogradsky CMU; 2013, Oct 1–6; Yalta, Ukraine. 2013; Abstracts: 386. Ukrainian.
- Khan A.S., Kniep B., Oelschlaeger T.A., Van Die I., Korhonen T., Hacker J. Receptor structure for F1C fimbriae of uropathogenic *Escherichia coli*. Infect Immun. 2000; 68: 3541–3547.
- Lakhtin V.M., Afanasiev S.S., Alyoshkin V.A., Nesvizhsky Y.V., Lakhtin M.V., Shubin V.V., et al. [Classification of lectins as universal regulator molecules of biological systems]. Announcer of Russian Academy of Medical Sciences. 2009; 3: 36–43. Russian.
- 12. *Lakhtin M., Lakhtin V., Alyoshkin A.* Lectin and enzyme relationships in microbiology. Intern. J. of Molecular and Clinical Microbiology. 2011; 1: 9–14.
- 13. *Lakhtin V.M., Lakhtin M.V., Pospelova V.V., Shenderov B.A.* Lectins of lactobacilli and bifidobacteria. II. Probiotic lectins of lactobacilli and as possible signal molecules regulating inter- and intrapopulating relationships between bacteria and between bacteria and the host. Microbial Ecology in Health and Disease. 2007; 19: 153–157.
- Lescar J., Loris R., Mitchell E., Gautier C., Chazalet V., Cox. V., et al. Isolectins I-A abd I-B of *Griffonia (Bandeiraea) simplicifolia*. Crystal structure of metal-free GS I-B(4) and molecular basis for metal binding and monosaccharide specificity. J. Biol Chem. 2002; 277: 6608–6614.
- Liu B., Cheng Y., Zhang B., Bian H.J., Bao J.K. Polygonatum cyrtonema lectin induces apoptosis and autophagy in human melanoma A375 cells through a mitochondriamediated ROS-p38-p53 pathway. Cancer Lett. 2009; 275: 54–60.
- 16. Liu B., Zhang B., Min M.W., Bian H.J., Chen L.F., Liu Q., et al. Induction of apoptosis by Polygonatum odoratum lectin and its molecular mechanisms in murine fibroblastoma L929. Biochem Biophys Acta. 2009; 1790: 840–844.
- 17. *Matsumoto I., Osawa T.* Purification and characterization of an anti-H (O) phytohemagglutinin of *Ulex europaeus*. Biochem Biophys Acta. 1969; 194: 180–189.
- Mukhopadhyay B., Martins M.B., Karamanska R., Russell D.A., Field R.A. Bacterial detection using carbohydrate-functionalised CdS quantum dots: a model study exploiting *E. coli* recognition of mannosides. Tetrahedron Lett. 2009; 50: 886–889.

- Muller W.E., Renneisen K., Kreuter M.H., Schroder H.C., Minkler I. The D-mannosespecific lectin from *Gerardia savaglia* blocks binding of human immunodeficiency virus type I to H9cells and human lymphocytes *in vitro*. J Acquir Immune Defic Syndr. 1988; 1: 453–458.
- 20. *Murphy L.A., Goldstein I.J.* Five alpha-D-galactopyranosyl-binding isolectins from *Bandeiraea simplicigolia* seeds. J Biol Chem. 1977; 252: 4739–4742.
- Podgorsky V.S., Kovalenko E.A., Karpova I.S., Sashchuk E.V., Getman E.I. [Extracellular lectins from saprophytic strains of bacteria of the genus *Bacillus* (review)] Applied Biochemistry and Microbiology. 2014; 50: 228–234.
- Podgorsky V., Kovalenko E., Karpova I., Sashchuk E., Getman E. New extracellular bacillus subtilis lectins with sialic acid specificity. J. Agricul. Sci. and Technology. 2014; 4: 541–546.
- Podgorsky V.S., Kovalenko E.A., Karpova I.S., Sashchuk E.V., Getman E.I. [Structural features and properties of extracellular lectin of strain *Bacillus subtilis* IMV B-7014]. Ukr Biochem Z. 2010; 82 (4): 1–10. Ukrainian.
- 24. Podgorsky V.S., Kovalenko E.A., Karpova I.S., Sashchuk E.V., Getman E.I. [Structural features of extracellular lectin of strain *Bacillus subtilis* IMV B-7014]. Dopovidi NANU. 2010; 9: 143–149. Ukrainian.
- 25. Podgorsky V.S., Kovalenko E.A., Karpova I.S., Sashchuk E.V., Getman E.I., Ruban T.O., et al. [Impact of lectin isoforms of *Bacillus subtilis* on viability of normal and malignant cells *in vitro*]. Microbiol Z. 2012; 74 (3): 3–9. Ukrainian.
- Podgorsky V.S., Kovalenko E.O., Karpova I.S., Sashchuk O.V. Bacillus subtilis soluble lectins. V Polish-Ukrainian Weigl Conference on Microbiology; 2013, May 23–25; Yuri Fedkovych National University. Ukraine, Chernivtsi: 2013; 50.
- 27. Pidgorsky V.S., Kovalenko E.O., Rybalko S.L., Sharykina N.I., Bardov V.G., Getman K.I., et al. [The obtaining method of extracellular lectins from bacteria of genus Bacillus and medical preparation on its basis for treatment sick people that were infected by HIV]. 2006; UA Patent No. 77477. Ukrainian.
- 28. *Pidgorsky V.S., Kovalenko E.O., Rybalko S.L., Sharykina N.I., Getman K.I.* [Medical preparation that contains bacterial lectin for treatment sick people that were infected by 1 and 2 types herpes viruses]. 2008; UA Patent No. 84928. Ukrainian.
- 29. *Pidgorsky V.S., Kovalenko E.O., Rybalko S.L., Sharykina N.I., Getman K.I.* [Medical preparation that contains bacterial lectin for treatment sick people that were infected by influenza virus]. 2008; UA Patent No. 83578. Ukrainian.
- 30. *Pidgorsky V.S., Kovalenko E.O., Rybalko S.L., Sharykina N.I., Getman K.I.* [Medical preparation that contains bacterial lectin for treatment sick people that were infected by hepatits C virus]. 2008; UA Patent No. 83305. Ukrainian.
- Podgorsky V.S., Kovalenko E.A., Simonenko I.A. [Bacterial lectins]. Kiev: Naukova Dumka; 1992. Russian.
- 32. Podgorsky V.S., Furtat I.M., Nogina T.M., Kovalenko E.A., Sashchuk E.V., Getman E.I. [Properties of lectins and cell biopolymers of nonpatogenic Corynebacterium]. Biopolymers and cell. 2011; 27(1): 40–46. Russian.
- 33. Sharon N., Lis H. Lectins. New York: Springer; 2007.
- Sharon N. Reflections: Lectins, carbohydrate-specific reagents and cell recognition molecules. J Biol Chem. 2007; 282: 2753–2764.

- 35. Spohr U., Hindsgaul O., Lemieux R.U. Molecular recognition. II. The binding of the Lewis b and Y human blood group determinants by the lectin IV of *Griffonia* simplificifolia. Can. J. Chem. 1985; 63: 2644–2652.
- 36. *Swanson M.D., Winter H.C., Goldstein I.J., Markovitz D.M.* A lectin isolated from bananas is potent inhibitor of HIV replication. J Biol Chem. 2010; 285: 8646–8655.
- 37. Wu A.M., Wu J.H., Chen Y.Y., Song S.C., Kabat E.A. Further characterization of the combining sites of Bandeiraea (Griffonia) simplicifolia lectin-I, isolectin A(4). Glycobiology. 1999; 9: 1161–1170.

Отримано 08.11.2016