Chapter 18

Lectin Systems Imitating Probiotics: Potential and Prospects for Biotechnology and Medical Microbiology

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1. Introduction

On the one hand, probiotics as microbial cellular preparations of usefulness for human include a lot of examples of successful applications supporting healthy status of organism. Majority of probiotics are represented by lactobacilli, bifidobacteria, and their mixtures [1]. Among them Acilact (consortium *Lactobacillus acidophilus* NK1 + 100_{ash} + K_3III_{24}), Lactobacterin (*L. plantarum* 8RA-3), Bifidin (*Bifidobacterium adolescentis* MC-42), Bifidumbacterin (*B. bifidum* N1), Biovestin (*B. adolescentis* + *B. bifidum*) and others are well-known probiotics produced and used in Russia (**Table 1**). These probiotics are based on probiotic strains from healthy adults gut (Collection of microorganism at G.N. Gabrichevsky Research Institute for Epidemiology & Microbiology [2]). However being of live cell origin, survival and metabolism of probiotics could not be reliably controlled, and theoretically in some cases originally probiotic bacteria have some risk to be changed towards decreasing useful activities and revealing negative features similarly to some relative pathogens. So search of non-cellular types of natural agents imitating probiotics is really important.

On the other hand, lectins as carbohydrate-binding/recognizing/sensitive proteins of non-immunoglobulin nature are multifunctional and multidomain (at least one type domain is CRD: carbohydrate binding at the level of aminoacid sequence), widely occur in nature [3 - 9], and can be specifically assembled to different soluble or not glycans, polysaccharides or glycoconjugates (GC) [glycoproteins, glycolipids, other glycol-non-proteins, any targets with exposed GC] in selected directions especially on solid or cell surfaces [10 - 15]. During assembling, lectin complexes: a) increase their multivalent and multifunctional recognition (more CBS: carbohydrate binding sites [CRD or epitopes in space], appearance of new types

of CBS and new targets are reached), b) form a dynamic partially reversible net system of lectin associates revealing carbohydrate recognition (the relatively changeable vector of resulting recognition by such a system can be evaluated by ordering a panel of carbohydrate targets according to their affinity to lectins). As a result, any lectin molecule in biological surroundings can be theoretically represented as: a) a lectin system (LS) of complexes and ensembles, b) a cascade of the directed assembling reactions, and c) a cascade system [16]. For example, complexes or oligomers of lectins or lectin-GC may be able to reveal new or modified carbohydrate/GC specificity, for example, in locations between subunits [14]. So lectin type cascades involving changeable originally the same molecules of lectins are possible.

Lectins are represented by more than 20 families and large groups involving in regulation of metabolism and widely used in biotechnology [5, 7, 8, 13, 15, 17]. Symbiotic microbial lectins are important regulators of relationships between microbes and eukaryotic macroorganisms [16]. However, among symbiotic lectins, PBL are the least studied recognition factors [8, 16, 18].

In 2004 probiotic bacterial lectins (PBL) including lactobacillar and bifidobacterial lectins (LL and BL) of human origin were firstly identified and preliminarily characterized by us [19]. The present study extend our knowledge concerning PBL as new class of natural symbiotic compounds. Such lectins may play important role in human superorganism in the regulation of inter- and intrapopulation relationships between bacteria and between bacteria and the host [20]. The data concerning lectins allow evaluation of important potential of PBL as cofunctioning factors produced by probiotics. The aim was to review our current study of PBL in aspects of their prospects for biotechnology and medicine.

2. Isolation and characterization of PBL

Criteria of choice of bacterial sources of PBL were:: a) probiotic lactobacilli and bifidobacteria, b) industrial strains, and c) consortium variants of increased antagonistic activities against reference microbial diagnosticums. Acilact corresponded to all these criteria. So LL isolated were represented as a combination of lectins of all ingredient strains of Acilact. Analogously, BL isolated included combination of lectins of strains MC-42 and N1. We studied lectins from probiotical lactobacilli and bifidobacteria, originally isolated from the healthy adults gut (**Table 1**).

Identification of PBL [20] was performed using a panel of biotinylated artificial polymeric linear water-soluble GC (www.lectinity.com). Advantages of such GC were homogenecity, multiple carbohydrate residues in side exposed positions (on polyacrylamide chain) similar to mucin glycan clusters or to simple carbohydrate antigen organization, and increased affinity of interaction due to polyvalent carbohydrate targets. The combined scheme of identification and isolation of PBL is presented in **Fig. 1**. The critical step of identification is isoelectric focusing of protein fractions in the slab of polyacrylamide gel followed by gel electric blotting to membrane. Immobilized lectins treated with biotinyl-GC were visualized by streptavidin-peroxidase conjugate in the presence of chemiluminescent substrate of

peroxidase using Dark Room of the BioChemi System (UVP, Calif.). Chemiluminescence kinetics was registered to optimise regime of PBL registration. The main positions of PBL were established (see **Table 2**). Lectins revealed in acidic region (within pI 4-4.5) of pH gradient were combined as acidic PBL (preparations aLL and aBL), and lectins revealed in basic region (within pI 7.6-8) were combined as basic PBL (preparations bLL or bBL). Additional PBL were identified as slightly acidic (within pI 5.1-6) [21] or approximately neutral. Artificial Mannan [GC as polyMan]- or (Mucin-like[GC as polyGalNAc])-binding PBL were rerpresented by LL (preferentially Mucin-like binding) and BL (preferentially Mannan-like binding). Combined preparations of LL (aLL or bLL) of Acilact were represented by contributions of the corresponding aLL or bLL of Acilact ingredient strains. Similarly, combined LB of strains MC-42 and N1 completed each other.

PBL were localized on the surface of bacteria (lactobacilli) within complexes which can be simply desorbed in the presence of LiCl (not NaCl). System of cell surface LL (as more protected) was represented by more extended panel of forms compared to secreted LL (as more dissociated and available to hydrolases of surrounding) into cultural fluid. Maximal forms of LL were obtained when boiled in the presence of sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (ME) (**Table 3**).

2.1. Isolation of PBL

Scheme of isolation of PBL is presented in **Fig. 1**. Being on the bacterial cell surface in complexes, PBL can be esially desorpted in vitro or in cultural fluids in the presence of chaotropic agents in combinations with surfactants (endogenic or exogenic) and chelate compounds. The way of isolation of active PBL is protected by the patent (in process). Procedure of PBL isolation needed approximately 3 days. As a result, PBL preparations were characterized as uncolor, transparent fluids, without smell, resistant to freezing.

1. Growth of bacteria in fluid medium.
2. Microfiltration and sterilization in *Steriflip* (Millipore).
3. Concentration and concentrate washing in *Centricon Plus-20* (Millipore).
4. Precipitation of concentrate with ice acetone.
5. Solubilization of precipitate in small volume.
6. Isoelectric focusing in slab of polyacrylamide gel in the presence of urea and saccharose.
7. Cutting out of lectins from the gel regions where acidic or basic PBL were identified*.
8. Extraction of PBL from gel.
9. Concentration and concentrate washing in phosphate buffer saline pH 7 (PBS).
10. Freezing and storing aliquots of PBL.

*Simultaneous identification of GC-binding PBL by blotting of a part of gel plate to membrane followed by membrane treating with GC-biotin and Streptavidin-Peroxidase.

Figure 1. Scheme of identification and isolation of PBL [5, 20].

No	Species*, strains	Previous names	Probiotics in Russia including s train as ingredient
1	*L. helveticus* NK1	*L. acidophilus* NK1	Acilact, Normospectrum, Polybacterin
2	*L. casei/paracasei* K_3III_{24}	*L. acidophilus* K_3III_{24}	Acilact, Normospectrum
3	*L. helveticus* 100_{ash}	*L. acidophilus* 100_{ash}	Acilact
4	*L. plantarum 8RA-3*	*L. plantarum 8RA-3*	Lactobacterin
5	*B. longum* MC-42	*B. adolescentis* MC-42	Bifidin
6	*B. bifidum* N1	*B. bifidum* N1	Bifidok, Bifidumbacterin

*[46, 47].

Table 1. Probiotic lactobacillar and bifidobacterial strains (ingredients of probiotics) used in our work

General properties: • *Original localization in ordered complexes within* cell surface layers; facilitated desorption into surroundings • *Molecular masses within* 52-80 kD • *System forms*: acidic [a] (within pI 3.7-4.5), slowly acidic (within pI 5.1-6), neutral (within pI 6.5-7.5) and basic [b] (pI 7.6-8) • *Contain exposed aromatic aminoacids*: Tyr (partially masked in different erxtent in aLL and aBL), Trp (preferentially in BL, Phe (some differences between aLL and aBL) • *Aggregation state* (preferentially for aL) • *Sensitivity to detergents (preferentially for bL)* • *Capability to adhesion on hydrophobic surfaces like polysterene and immobillon P* (aL > bL) • *Contain ions* Ca, Mg	
Acidic LL: major 58-59 kD minors 60-62 and 53-55 kD pI 3.8-4 (2 bands) $(D_{350} – D_{400})/D_{240}$= 46.3	*Acidic BL*: Majors and minors 56-57, 53-54, 60-64 kD pI 3.7-4.2 (1 band + 2 dublet bands) $(D_{350} – D_{400})/D_{240}$ = 66.7
Basic LL: 62-80 kD , pI 7.6-8 $(D_{350} – D_{400})/D_{240}$ = 33.8	*Basic BL*: 58-62; 52-54 kD; pI 7.6-8 $(D_{350} – D_{400})/D_{240}$ = 33.2

D= optical density.

Table 2. Physicochemical and biochemical properties of PBL [5, 20, 22]

Sources of PBL	Specificity to polymeric GC	Positions of PBL bands, pI*	Intensity of PBL**
Protein concentrates			
B. adolescentis MC-42	GalNAc- Man(6-P)- Gal(3-Sulfate)-	7.5-8 6; 8 4-5; 7	4+/3+ 3+/+ 4+/+
B. gallinarum GB***	GalNAc- Man(6-P)- Gal(3-Sulfate)-	8 6.5; 7.5-8 7	2+ 2+/+/3+ 2+
B. bifidum N1	GalNAc- Man(6-P)- Gal(3-Sulfate)-	7-7.5 8 4-5	2+ 2+ 4+
LiCl-cell surface extracted protein concentrates			
L. acidophilus 100_{ash}	GalNAc- Man(6-P)- Gal(3-Sulfate)-	5.1-7-7.5;8 6.5 5; 7	+/4+/3+ 2+ +/3+
L. acidophilus NK1	GalNAc- Man(6-P)-	8 6;6.5;7	2+ +/2+/+
(SDS+ME)-treated proteins of concentrate fractions			
Acilact	GalNAc- Man(6-P)-	4.5-5.5;5.8;6.3 5.5-6.5	3+/3+2+ 2+/3+/2+
L. acidophilus 100_{ash}	GalNAc- Man(6-P)- Gal(3-Sulfate)-	5.8;6.2 6-7 5; 7	+/+ 2+/+ +/3+
L. acidophilus NK1	GalNAc- Man(6-P)-	4.5-5;5.7;6.2 5;6-6.8;7-8	3+/2+/+ 2+/2+/4+

*Isoelectric points (pI) according to isoelectric focusing in PAA gel in gradient of pH 4 - 8; ** in scale "+" - "4+" (relative chemiluminescence of complex PBL-b—Streptavidin-Peroxidase in the presence of chemiluminescent substrate of peroxidase). *** strain from chicken gut. SDS: sodium dodecyl sulfate, ME: 2-mercaptoethanol.

Table 3. Identified PBL of different types [20].

The main physicochemical and biochemical properties of PBL are presented in **Table 2**. As it can be seen from the **Table 2**, PBL are relatively hydrophobic proteins and can be presented in aggregated forms with partially exposed aromatic aminoacid residues (especially controlled for Tyr and Trp). Protein stability of PBL needed the presence of cocktail of protease inhibitors ("*Complete*", R & D). Increased disappearance of bBL upon storing in glass tubes (compared to polypropylene tubes) for a long time was observed (increased sorption on glass walls is possible). PBL contained cations of metals. For example, major

forms of PBL of *L. helveticus* NK1 (strain as dominated contributors of LL into Acilact) contain approximately 2 Ca^{2+} in molecule. Fluorescent properties of PBL (especially in case of BL) are increased in PBL complexes including endogenic exopolymers.

Aforementioned data allow preliminary classifications of PBL [5, 7, 16]. Currently, PBL can be considered as: originally surface proteins of recognition, Ca^{2+} (and other metal cation)-containing and binding proteins, relatively (random structure)-organized (decreasing of randomly ordered structure in complexes as refolding recognition process), preferentially originally mono- or bivalent (one CBS in polypeptide) low sensitive haemagglutinins (similar to pan-agglutinins), with capability to create complexes, oligomeres and aggregated particles, members of functional superfamilies.

2.2. Biological properties of PBL

PBL imitate the following general main activities of probiotics: antimicrobial, immunocorrecting, ssupporting consortium, stabilizing healthy status in communicative directions "Microbes - Microbes" and "Microbes - Host". In addition, PBL reveal unique properties which complete probiotics to synbiotics and extend spectrum of useful activities in combinations "Probiotics + PBL" (see below).

PBL are represented by four LS (**Table 1).** Among them LL and LB (acidic and basic) were isolated and studied by us in detail. In addition, in case of slowly acidic LL it was suggested their potential cofunctioning to oxidase-reductase system within potential lactobacillus consortium of Acilact strains and *L. plantarum* 8RA-3 [21]. The role of such LL may be in regulation of protection of probiotic consortium in biotopes against peroxide stress. Examples of regulation of oxidoreductases with lectins are well documented [15]. Mean time, the role of neutral LL is still unclear.

2.2.1. Interactions between PBL and GC [14, 17, 19, 20, 22-24]

Major forms of soluble PBL are represented mainly as molecules and their complexes with one CBS. Such PBL forms needed hydrolase treated red cells for visualization of haemagglutination reaction. In haemagglutination reaction (*Clostridium perfringens* sialidase-treated human AII-blood group erythrocytes) interaction between PBL and GC was as approximately equimolar (1 : 1, M/M).

We identified different lectins secreted by lactobacilli and bifidobacteria using a panel of GC and mainly three methods including: a) dot-blotted supernatant concentrates on Immobillon-P membrane (Millipore), b) proteins blotted after isoelectric focusing supernatant concentrated protein fractions in polyacrylamide plate, c) proteins sorpted on sialidase (or trypsin)-treated human AII-red cells [5, 22 - 25].

For identification of lectins among extended panel of lactobacilli and bifidobacteria strains we used GC (0.5-5 mkg/ml, PBS) containing multiply exposed side carbohydrate residues on biotynylated (b) or not polyacrylamide (PAA) chain (www.lectinity.com):

- Fucα1- [α-L-Fucan-like],
- Galβ1- [β-D-Galactan-like],
- Gal(3-Sulfate)β1- [3-HSO_3Galβ1- ;β-D-Galactan-3-Sulfate polymer],
- GaNAcα1- [Tn-like antigen containing polymer],
- GalNAcα1,3Galβ1- [A_{di} as (AII-blood group substance)-like containing polymer],
- GalNAcα1,3GalNAcβ1- [Fs as (Forssman antigen)-like containing polymer],
- GalNAcα1,3GalNAcα1- ,
- Galα1,3GalNAcα1- [$T_{\alpha\alpha}$-like antigen containing polymer],
- GalNAcβ1- [desialylated Mucin-like],
- Galβ1,4GlcNAcβ1- [poly(LacNAc)-containing mucin-like],
- GlcNAcβ1- [soluble linear Chitin-like],
- Manα1- [α-D-Mannan-like],
- Man(6-phosphate)α1- [6-H_2PO_3Manα1-polymer; α-D-PhosphoMannan],
- (MurNAc-L-Ala-D-isoGln)β1- [MDP-; Muramyldipeptide containing polymer; bacterial Peptidoglycan-like],
- Rhaα1- [α-L-Rhamnan-like].

The whole resulted chemiluminescent pictures of LL and BL separated by isoelectric focusing followed by blotting were distinct and needed individual optimized regimes of registration. It is seen from the **Table 3** that: a) the pictures of PBL are unigue and depended on strain origin, b) dominated PBL types are revealed as mucin- and/or Mannan-binding; b) PBL of probiotic consortium include PBL of ingredient strains. Mannan-binding lectins of *L. plantarum* 8RA-3 possessed increased intensities of chemiluminescence [19]. These data were supported by study of PBL specificity to GC in haemagglutination reaction [5, 23]. Dissociation of PBL-(hydrolase-treated human AII-red cells) agglutinates was observed in the presence of 0.5-1 mkg/ml of GC. Effectivenes of GC was decreased in the order: poly(GalNAc) or Mannan > Galactan >> Chitin-like polymer (no influence).

In other seria of experiments we extended panel of probiotic bacteria and extended panel of GC to identify new PBL types using dot-blotting technique [24, 25]. It was shown that PBL of lactobacilli and bifidobacteria are capable to discriminate GalNAc-containing GC (GalNAc residues as exposed, internal/masked, or dublicated) glycoantigens A_{di}-, Fs-, or T_n-depending on strain origin. No binding of PBL to $T_{\alpha\alpha}$ was observed. PBL also discriminated artificial peptidoglycan, mannans and mucins. Due to PBL revealing as LS [16] when two or more PBL forms (major and minor ones) vary on specificity, similarity (identical part of mosaic of the same specificity) and differences (the whole mosaic as unique, ranging intensity of components with the same specificity, some components which simultaneously recognize two types of target GC) between recognizing potential of species and genus of lactobacilli and bifidobacteria can be established.

Using dot-blotting technique, at least 7 types of LS were identified for extended panel of lactobacilli and bifidobacteria which occur in human gut. Among these, LS were represented by lectins which especially significantly recognized α–D-Mannan (phosphorylated or not; yeast-like), α-L-Fucan (algal-like), peptidoglycan (bacterial-like),

mucins (mammalian gut-like); antigens T_n and Forssman, blood group AII substance. Such lectins were identified as mosaic within bacterial mainly acidic protein massive.

Aforementioned data on interaction between PBL and GC indicate that PBL may serve as additional important functional characteristic. The latter can serve the basis to study biotope metabolic relationships involving probiotic bacteria as antagonistic to opportunistic microorganisms in keeping healthy biotope status; and to construct cofunctioning systems of PBL together with yeast and higher plant ingredients.

Antimicrobial activities of PBL against clinical microbial strains [21, 26-32] included:

- Growth inhibition;
- Involving biodegradation (proteolysis) (LL > BL);
- Synergistic action (LL + BL: against staphylococci [effectiveness: LL > BL]; BL + LL: against microfungi [effectiveness: BL > LL]; BL + antibiotics: against *Candida* species [possibility to decrease effective work doses of antibiotics]);
- Action as cascades (action of aPBL followed by action of bPBL);
- Concurent use of resources of pathogens during their different live cycle steps (wrong essembling of biofilms of pathogens, choice and switching of metabolome nets, increased degradation of pathogen constructions including their lysis).

The following general comments on antimicrobial action of PBL should be noted. The action of PBL is directed against colorectal and urogenital clinical strains from human biotopes. PBL act as the members of new class of biofilm destructors [27]. Anti-*Staphylococcus* and anti-*Candida* action reveal multistep synergism in space (different regions of action of aLL and bLL, aBL and bBL, aLL and aBL, antibiotic-like and lytic actions) and in time (earlier action as antibiotic-like, later lytic action of aPBL followed by lysis by bPBL). It takes place multisynergism of anti-*Candida* action between PBL and antibiotics (azoles, amphotericin B, nystatin). Taken together, PBL imitate anti-*Staphylococcus* and anti-*Candida* activities of probiotic lactobacilli and bifidobacteria [33, 34] and can be potentially used for treatment of candidoses and staphylococcoses.

It should be also noted that PBL possess advantages compared to other antimicrobials: prolonged action; cascade synergistic action, low subcytoagglutinating doses; non-dependence on antibiotic types (upon therapy) [probiotics delivered can be inactivated by some antibiotics]. In addition, ketokonazol and some other antibiotics are poorly soluble in PBS that decreases their effectiveness and control.

2.2.2. Activities of PBL in respect of potential probiotic compartment of biotope [35, 37, 38]

PBL reveal a spectrum of activities in respect of populations of lactobacilli isolated from the same biotope. Results indicate that LL support healthy status of normoflora in biotope due to realization of supervisor signal functions of PBL. It is expected that when delivered, PBL increase synbiotic compartment of biotope against potential pathogenic compartment (in addition to other positive events in direction "Microbiocenoses - Host").

2.2.3. Other biological activities of PBL

Activities of PBL in respect of cells of mammalian protection systems [22, 27]:

- Inducing production of TNF-α by human periphery blood lymphocytes;
- Modulation of peritoneal macrophage migration in manner which is differed from action of GC;

Predicted PBL activities based on similarities to symbiotic bacterial lectins [16], other lectins and probiotics:

- As possible ingredients of drug forms in cases of colorectal alterations (potential effectiveness: BL > LL), or urogenital alterations (potential effectiveness: LL > BL).
- Direct antitumor action: against changed human cell systems similarly to PBL action against eukaryotic pathogens as xenoagents in organism (potential effectiveness: BL > LL); througph increased affinity of PBL to Poly(LacNac) as potential tumor antigens [14] (potential effectiveness: LL > BL);
- Against protozoan pathogens (like action against another type of eukaryotic pathogens - microfungi);
- Against viruses (like Acilact action against rotavirus infection of children; similar to Mannan-binding phytolectins possessing activity against HIV-1);
- Intracellular sorting into organells and vesicules [due to capability of PBL to recognize poly(Man-6-P) within targets, similar to animal Man-6-P-binding lectins)];
- Biocompatibility and synergism of LL and/or BL together with other probiotic microbial lectins as antimicrobials;
- Biocompatibility and synergism of PBL to other type antimicrobials possessing distinct mechanisms of action;
- The possible forming additional antimicrobial pool as PBL fragments in the presence of host and microbial hydrolases of surroundings in biotopes.

3. Conclusion

All aforementioned data support wide potential of PBL for industrial and medical biotechnology.

The following main prospects of applications of PBL can be underlined:

For cell cultures [autostimulators, supporting probiotic bacterial cultures: mixed or not, in the presence of pathogen, etc.],

In constructing bioadditives, anti-infectives and drug forms [system drugs of synergistic and selective action as antipathogenic agents, and as factors supporting probiotic compartment in biotopes];

In diagnostics [microassays; for typing clinical pathogen strains; for detecting altered anormal surface and metabolome net of pathogenic significance] [28, 29, 32],

In constructing of cascade biosensors based on LS-organization of PBL [for monitoring biotope healthy balance, for screening strains and their mixtures especially on solid surfaces like sensibilized membranes, polysterol or polypropelene;

In constructing predictable lactobacilli- and bifidobacteria-based consortia as potentially probiotics-like, constructing synbiotic consortia [37, 38]:

- keeping metabolome status;
- switching (on/off) microbial nets and cascades;
- controlling microbiocenosis functioning; providing cell teaching;
- factors in constructing of beneficial microbiocenoses;
- directed antipathogen action by changing ontogenesis of pathogens) [27];
- helpers in building cell and cytokine-like gradients [23];
- synergistic and synbiotic factors in mixed cultures of microorganisms or in host biotopes [32];
- stabilizers of poorly growth probiotic microorganisms [35, 36];
- co-functioning with other PBL-like and non-PBL antimicrobials produced by probiotic compartment of biotope (bacteriocins, antimicrobial peptides and biosurfactants) [22, 39]
- co-functioning with human cytokines (PBL as cytokine inducers), defensins, antibiotics (synergism), antibodies [9, 39, 40];
- synergism of PBL signaling and signal proteinases/oligopeptidases and (oligo)peptides of surrounding;
- screening, selection and typing of strains;
- ingredients of both free cell drug ointments and cosmetic creams (improving formulas) [39, 41];
- in recombinant lectin technologies [17];
- carriers for drug delivery, carriers of low molecular weight highly hydrophobic heterocyclic effectors (some antibiotics, chemotherapeutic antitumor agents, apoptose inducers, etc.) [3, 17];
- ingredients of functional bioadditives [41];

Upon chemotherapy and radiotherapy of tumors to support healthy status of organism [36, 42]

In system drug therapy when added PBL (LL and/or BL) will modulate whole spectrum of system drug activities;

In landscape microecology and architectuire of microbiocenoses (PBL as the direct participants and organizers of landscapes) [32].

It is clear that solid or cell surfaces are of preferential importance for any directed assembling initiated by PBL (increased accumulated interphased concentrations of reactants, initiating or triggering assembling on immobilized first components of cascades, achievement of maximally long and asymmetric products). That is why PBL within pore PAA hydrophilic gels or membranes (Durapore membranes as [multi]layer microaccumulators), immobilized PBL on PVDF membranes (Immobillon P) or polysterene microplates and latex particles are of especial perspectiveness.

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Appendix

List of abbreviations

a – acidic

b – basic

CBD – carbohydrate binding domain

CBS – carbohydrate binding site

D – optical density

BL bifidobacterial lectins

LL lactobacillar lectins

LS lectin system(s)

PBL – probiotic bacterial lectins

GC – glycoconjugate(s)

PBS – phosphate buffer saline pH 7

4. References

[1] Shenderov BA (2008) [Functional Foods and their role in prophylaxis of metabolic syndrome (in Russian)]. Moscow: DeLi Print, 319 pp. ISBN 978-5-94343-166-1.

[2] Aleshkin VA, Amerhanova AM, Pospelova VV, Afanasyev SS, Shenderov BA (2008) History, Present Situation and Prospects of Probiotic Research Conducted in the G.N. Gabrichevsky Institute for Epidemiology and Microbiology. Microb Ecol Health & Dis 20: 113-115.

[3] Lakhtin VM (1987) [Lectins for Investigation of Proteins and Carbohydrates (in Russian)]. In: Vsesoyuzniy Institut Nautchnoy I Tehnitcheskoy Informatsii, Moskva, Itogi Nauki I Tehniki, Seriya Biotehnologiya, Vol 2 (Klyosov AA, ed) [VINITI, Moscow, Reviews of Science and Technique, Series Biotechnology, Vol 2 (Klyosov AA, ed) (in Russian)]: 290 p. ISSN 0208-2330.

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[4] Lakhtin VM (1989) [Lectins and Aspects of Their Study (in Russian)]. Mikrobiologitcheskiy Zhurnal (Kiev) 51; No 3: 69 – 74. ISSN 0201-8462.

[5] Lakhtin VM, Lakhtin MV, Pospelova VV, Shenderov BA (2007) Lectins of Lactobacilli and Bifidobacteria. II. Probiotic Lectins of Lactobacilli and Bifidobacteria as Possible Signal Molecules Regulating Inter- and Intrapopulation Relationships Between Bacteria and Between Bacteria and the Host. Microb Ecol Health & Dis 19: 153 - 157.

[6] Korsun VF, Lakhtin VM, Korsun EV, Mitskonas A (2007) [Phytolectins (in Russian)]. Moscow: Practical Medicine, 288 p. ISBN 5-98811-077-0.

[7] Lakhtin VM, Afanasiev SS, Aleshkin VA, Nesvizskyi UV, Lakhtin MV, Shubin VV, Cherepanova YV, Pospelova VV (2009) [Classification of lectins as universal regulator molecules of biological systems (in Russian)]. Vestnik Rossiiskoy Academii Meditsinskih Nauk No 3: 36-43. ISSN 0869-6047.

[8] Lakhtin V, Lakhtin M, Aleshkin V (2011) Lectins of Living Organisms. The Overview. Anaerobe 17: 452 - 455. DOI: 10.1016/j.anaerobe.2011.06.004.

[9] Lakhtin MV, Karaulov AV, Lakhtin VM, Alyoshkin VA, Afanasyev SS, Nesvizskyi UV, Afanasyev MS, Vorapaeva EA, Alyoshkin AV (2012) [Lectin – glycoconjugate systems in human organism (in Russian)]. Immunopathology, Allergology, Infectology No 1: 27-36. ISSN 0236-297X.

[10] Lakhtin VM (1991) [Lectins in the Investigation of Receptors (in Russian)]. Uspekhi Khimii (Moskva) 60: 1777 - 1816. ISSN 0044-460X.

[11] Lakhtin VM (1992) [Specificity of Microbial Lectins (in Russian)]. Prikladnaya Biokhimiya i Mikrobiologiya (Moskva) 28: 483 - 501. ISSN 0555-1099.

[12] Lakhtin VM (1994) Lectin Sorbents in Microbiology. In: "Lectin - Microorganism Interactions". Doyle RJ, Slifkin M, eds. New York: Marcel Dekker. 1994. pp. 249 - 298. ISBN 0-8247-9113-4.

[13] Lakhtin VM (1994) [Molecular organization of lectins (in Russian)]. Molekulyarnaya Biologiya (Moskva) 28: 245-273. ISSN 0026-8984.

[14] Lakhtin VM (1995) [Use of Lectins in the Analysis of Carbohydrate Moieties of Glycoproteins and Other Natural Glycoconjugates (in Russian)]. Biokhimiya (Moskva) 60: 187-217. ISSN 0320-5725.

[15] Lakhtin MV, Lakhtin VM, Aleshkin VA, Afanasiev SS, Aleshkin AV (2010) [Lectins and Enzymes in Biology and Medicine (in Russian)]. Moscow: "Dynasty" Publishing House, 496 pp. ISBN 978-5-98125-076-7.

[16] Lakhtin M, Lakhtin V, Aleshkin V, Afanasiev S (2011) Lectins of Beneficial Microbes: System Organization, Functioning and Functional Superfamily. Beneficial Microbes 2: 155 – 165. DOI: 10.3920/BM2010.0014.

[17] Lakhtin VM (1989) [Biotechnology of Lectins (in Russian)]. Biotechnologiya (Moskva) No 6: 676-691.

[18] Lakhtin VM, Aleshkin VA, Lakhtin MV, Afanasiev SS, Pospelova VV, Shenderov BA (2006) [Lectins, Adhesins and Lectin-like Substances of Lactobacilli and Bifidobacteria

(in Russian)]. Vestnik Rossiiskoy Academii Meditsinskih Nauk 1: 28-34. ISSN 0869-6047.

[19] Lakhtin VM, Pospelova VV, Lakhtin MV, Medvedkova NA, Bovin NV (2004) [Interaction of components of cultural fluids of lactobacilli and bifidobacteria to synthetic analogs of polysaccharides (in Russian)]. Proceedings of the International Conference "Probiotics, Prebiotics, Synbiotics and Functional Foods. Modern State and Prospects" (June 2-4, 2004, Moscow) Moscow, 2004: 28.

[20] Lakhtin VM, Lakhtin MV, Pospelova VV, Shenderov BA (2006) Lactobacillus and Bifidobacterial Lectins as Possible Signal Molecules Regulating Intra- and Interpopulation Bacteria - Bacteria and Host - Bacteria Relationships. Part I. Methods of Bacterial Lectin Isolation, Physicochemical Characterization and Some Biological Activity Investigation. Microb Ecol Health & Dis 18: 55 - 60.

[21] Lakhtin M, Aleshkin V, Afanasiev S, Pospelova V (2011) Three Protective Lectin Systems In Probiotic Lactobacillus Consortium (poster 360). Proceedings of the 4th Congress of European Microbiologists (26-30 June, 2011, Geneva, Switzerland). Program Book: P. 94. CD-ROM Abstracts, "L" Names.

[22] Lakhtin MV, Alyoshkin VA, Lakhtin VM, Nesvizhsky YV, Afanasyev SS & Pospelova VV (2010) [The Role of Lectins from Probiotic Microorganisms in Sustaining the Macroorganism (in Russian)]. Vestnik Rossiiskoy Academii Meditsinskih Nauk 2: 3-8. ISSN 0869-6047.

[23] Lakhtin VM, Lakhtin MV, Korsun VF & Shenderov BA (2008) [Mutual Potential of Lectins of Probiotic Microorganisms and Fungi in Conditions of Organization and Functioning of the Model Eukaryotic Cell Biofilms - for Further Use in Clinical Practice within Phytocompositions (in Russian)]. Praktitcheskaya Fitoterapiya 2: 11-17. ISBN5-88010-096-0.

[24] Lakhtin VM, Dykhal YI, Lakhtin MV, Cherepanova YV, Pospelova VV, Shenderov BA (2009) New Lectin Systems in Cultural Fluids of Probiotic Strains of Lactobacilli and Bifidobacteria Capable to Discriminate Glycoantigens Containing GalNAc. Gastroenterologia Sankt-Peterburga [Gastroenterology of Sankt-Petersburg] 4: A14-A15. ISSN 1727-7906.

[25] Lakhtin V, Alyoshkin V, Lakhtin M, Afanasyev S (2009) Glycoconjugates in Discrimination of Glycoconjugate Recognition Systems of Probiotic Microorganisms. New Potential Keys for Strains and Glycometabolome Typing. Glycoconjugate Journal. 26: 876.

[26] Lakhtin MV, Lakhtin VM, Aleshkin VA, Afanasiev SS, Korsun VF (2010) [Phyto- and Probiotic Lectins – Synergistic Antipathogens (in Russian)]. Praktitcheskaya Fitoterapiya 1: 5-11. ISBN5-88010-096-0.

[27] Lakhtin M, Aleshkin V, Lakhtin V, Afanasiev S, Pozhalostina L, Pospelova V (2010) Probiotic Lactobacillus and Bifidobacterial Lectins Against *Candida albicans* and *Staphylococcus aureus* Clinical Strains: New Class of Pathogen Biofilm Destructors. Probiotics & Antimicrobial Proteins 2: 186-196. DOI: 10.1007/s12602-010-9046-3.

[28] Lakhtin MV, Bajrakova AL, Lakhtin VM, Alyoshkin AV, Afanasiev SS, Aleshkin VA (2011) [Microbiocenosis Model "Pathogen – Probiotic lectins" for Monitoring Primate Biotope Dysbioses (in Russian)]. Proceedings of the 2nd International Scientific Conference "Fundamental and Applied Aspects of Medical Primatology" (August 8-10, 2011, Sochi, Russia). Vol 1. Sochi: 88-92.

[29] Lakhtin MV, Bajrakova AL, Lakhtin VM, Afanasiev SS, Aleshkin VA, Korsun VF (2011) [Probiotic Lectins of Human in Protection against Dysbioses in Different Human Biotopes (in Russian)]. Praktitcheskaya Fitoterapiya (Moskva) 1: 4-13. ISBN5-88010-096-0.

[30] Lakhtin MV, Lakhtin VM, Aleshkin VA, Bajrakova AL, Afanasiev SS, Korsun VF (2011) [Phyto- and Probiotic-Analog Therapy of Microfungal Infections: Theory and Practice Potential in the Development (in Russian)]. ARS MEDICA 15: 183-187. ISSN 2220-5497.

[31] Lakhtin M, Lakhtin V, Bajrakova A, Aleshkin A, Afanasiev S, Aleshkin V (2012) Interaction of Probiotic Bacterial Lectins to *Candida* Species. Proceedings of the VIII International Conference "Sciences and Technology: Step in Future (Feb. 27 – March 5, Prague, Chechia)". – Vol. 29: 34 - 41. Materiály VIII mezinárodní vědecko - praktická conference «Věda a technologie: krok do budoucnosti - 2012». - Díl 29: 34 – 41. Biologické vědy: Praha. Publishing House «Education and Science». ISBN 978-966-8736-05-6.

[32] Lakhtin VM, Lakhtin MV, Afanasiev SS (2012) [The fight for the space and resources between probiotic and relatively pathogenic compartments in potential biotope: lectin type imitators of probiotics against eukaryotic pathogens – importance for biotechnology (in Russian)]. Proceedings of the VIII International Conference "Key questions in Modern Science" (April 17 – 25, Sofia, Bulgaria). Sofia, Vol. 28: 28 – 33. Материали за 8-а международна научна практична конференция, «Ключови въпроси в съвременната наука», - 2012. Том 28: 28 – 33. Биологии. Селско стопанство. София. «Бял ГРАД-БГ» ООД. ISBN 978-966-8736-05-6.

[33] Krisenko OV, Sclyar TV, Vinnikov AI, Kiryukhantseva IM (2012) [Features of antagonistic activity of lactic acid bacteria in respect of microfungi (in Ukrainian)]. Proceedings of the VIII International Conference "Key questions in Modern Science" (April 17 – 25, Sofia, Bulgaria). Sofia, Vol. 28: 18-23. [Материали за 8-а международна научна практична конференция, «Ключови въпроси в съвременната наука», - 2012. Том 28. Биологии. Селско стопанство. София. «Бял ГРАД-БГ» ООД]. ISBN 978-966-8736-05-6.

[34] Lazarenko L, Babenko L, Shynkarenko-Sichel L, Pidgorskyi V, Mokrozub V, Voronkova O, Spivak M (2012) Antagonistic Action of Lactobacilli and Bifidobacteria in Relation to *Staphylococcus aureus* and Their Influence on the Immune Response in Cases of Intravaginal Staphylococcosis in Mice. Probiotics & Antimicro Prot 4: 78 – 89. DOI 10.1007/s12602-012-9093-z.

[35] Lakhtin VM, Bajrakova AL, Lakhtin MV, Belikova YV, Afanasyev SS, Aleshkin VA (2012) [Regulating properties of the human probiotic bacterial consortium lectins: Screening, modulation and selection of normoflora of *Lactobacillus* populations from the same biotope (in Russian)]. Proceedings of the VIII International Conference "Days of Science". Prague, Vol. 74: 38 – 44. Materiály VIII mezinárodní vědecko - praktická conference «Dny vědy - 2012». - Díl 74: 38 – 44. Biologické vědy: Praha. Publishing House «Education and Science» s.r.o. ISBN 978-966-8736-05-6.

[36] Lakhtin M, Lakhtin V, Bajrakova A, Afanasyev S, Alyoshkin V (2011) Human Biotope Probiotic Bacterial Lectins as Signal System Supporting Biotope Healthy Balance. Immunology 135, Suppl. 1:111.

[37] Lakhtin MV, Lakhtin VM, Cherepanova YV, Pospelova VV, Afanasyev SS, Alyoshkin VA (2011) Ranging qualities of industrial ingredient probiotic strains of human bifidobacteria and lactobacilli to predict new probiotic formulas. Proceedings of International Conference "Modern Achievements of Biotechnology" (June 21-23, 2011, Stavropol, Russia). Part 2. Division "Bioadditives". Moscow: NOU "Education Scientidic Technical Center of Milk Industry", 49 – 51.

[38] Lakhtin VM, Lakhtin MV, Agapova YV, Belikova YV, Kulakova YV, Afanasyev SS, Alyoshkin VA (2012) Advantadges of the probiotic "Acilact" compared to ingredient strains using algorithmic ranges of qualities. Proceedings of the VIII International Conference "Scientific Space of Europe - 2012" (April 7-15, Przemyśl. Poland). Przemyśl, Nauka i studia. Vol. 32: 50 – 57. Materiały VIII Międzynarodowej naukowi-praktycznej konferencji «Naukowa przestrzeń Europy - 2012» Vol. 32. – P. 50-57. Nauk biologicznych.: Przemyśl. Nauka i studia. ISBN 978-966-8736-05-6.

[39] Lakhtin VM, Afanasiev SS, Aleshkin VA, Nesvizhsky YV, Pospelova VV, Lakhtin MV, Cherepanova YV, Agapova YV (2008) [Strategical Aspects of Constructing Probiotics of the Future (in Russian)]. Vestnik Rossiiskoy Academii Meditsinskih Nauk 2: 33-44. ISSN 0869-6047.

[40] Lakhtin VM, Afanasyev SS, Aleshkin VA, Nesvizhsky YV, Pospelova VA (2008) [Nanotechnologies and perspectives of their application in medicine and biotechnology (in Russian)]. Vestnik Rossiiskoy Academii Meditsinskih Nauk No 4, 50-55. ISSN 0869-6047.

[41] Lakhtin MV, Lakhtin VM, Aleshkin AV, Bajrakova AL, Afanasiev SS, Aleshkin VA, Korsun VF (2012) [Probiotic Lectins – Ingredients of Biopreparations, Bioadditives and Drug Forms (in Russian)]. Praktitcheskaya Fitoterapiya (Moskva) No 1: ISBN5-88010-096-0.

[42] Lakhtin MV, Aleshkin VA, Lakhtin VM, Nesvizhsky YV, Afanasiev SS, Pospelova VV (2012) [Prospects of Lectins of Probiotics in Chemotherapy (in Russian)]. Proceedings of the VIII International Conference "Days of Science" (March 27 – April 5, Prague, Chechia). Prague, Publishing House «Education and Science» s.r.o. Vol. 74: 6 – 10. Materiály VIII mezinárodní vědecko - praktická conference «Dny vědy - 2012». - Díl 74:

6 – 10. Biologické vědy: Praha. Publishing House «Education and Science» s.r.o. ISBN 978-966-8736-05-6.

[43] Botina SG (2011) [Molecular biological approaches in selection of bacterial cultures upon creation of inoculi for biotechnology (in Russian)]. Thesis, DSc (Biotechnology). Moscow: 280 pp.

[44] Subbotina ME (2009) [Development of method of gene typing of bifidobacteria using bi-locus sequenation to identify species of strains (in Russian)]. Thesis, PhD (Genetics). Moscow: 122 pp.

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