Mucosal innate immunity involves system “Probiotic lectins—Glycoconjugates” against microbial pathogens

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The paper is overview of mainly own works. It is devoted to new insights of mucosal innate immunity involving lectin systems of probiotic bacteria (LSPB) recognizing glycoconjugates (GC). LSPB and probiotic phytolectins supporting human health (separately or in combinations with other antimicrobials and antipathogen factors) are important contributors to biotope distant protection against pathogens. Being metabolomebiotics, LSPB form reversible complexes to natural and artificial polymeric polyvalent GC that allows structure-functional building directed on duty network of cascades for predicted replies. Probiotic molecular-cellular network supersystem LSPB—GC serves as important synergistic one contributing to biotope human and microbiocenosis antipathogen resistance. This network influences mucosa pores, affine texture, and microecological redistribution in organism. Mucosal innate immunity LSPB-based concept and strategies against microbial and viral pathogens are proposed. Recognition activities of artificial polymeric GC and LSPB were compared. Prospects of antipathogen lectin-containing systems cofunctioning to antibodies, cytokines, enzymes, phytolectins and antibiotics are underlined.

Keywords: mucosa; innate immunity; probiotic recognition, probiotic lectin system; glycocojugates; microbiocenosis; probiotic bacteria; pathogens; tumor like cells.

1. Introduction

Strategic aspects of medical microbiology and biotechnology at present time and future are important goals for investigators [1-3]. Lectins are widely distributed and have microecological significance [4, 5]. Lectins reversibly recognize glycoconjugates (GC) including glycoproteins and glycopeptides, proteoglycans and glycosaminoglycans, (lipo)polysaccharides, other modified glycopolymers. Carbohydrate moieties and extended glycotopes (bi/oligosaccharides, glycopeptides, others) of GC serve additional targets which cofunction to lectin systems (LS). LS interacting to GC systems (GCS) form multifunctional directed network of LS—GCS complexes [4, 5]. LS and GCS participate in regulation of biorecognition systems such as Antibodies(Ab)—Antigens, Enzymes—(Their modulators), Cytokines—Cells, (Defensins and other antimicrobial oligo/polypeptides)—Microbes, Multifunctional cell receptors of lectin type, (Protein hormones)—Receptors, Complement and Blood clotting systems, others [2, 6].

Thus, LS serve as universal widely distributed recognition helpers of intermolecular, intercellular and supracellular functioning. They are contributors into tissue (LS of homing) and organ (LS as result of unit vector summary of all type mixed LS) tropisms. Lectins are cofunctioning elements infolving Fc-binding receptors, intramolecular special site of enzymes of carbohydrate metabolism (the presence of lectin type site which orientates target towards catalytic centre). Being evolutionary ancient recognition factors, LS serve as evolutionary basis for further functional overbuilding recognition systems. LS function as cofunctioning systems of the second plane (according to lower evolutionary hierarchy status) providing comfort to other evolutionary advanced recognition mechanisms. LS serve as adaptors of assemblings possessing autoregulating activities, organizers of active architectures [6, 7]. During interactions of lectins and their targets, new lectin activities and modulation of previous ones can take place [2, 6].

LSPB function as synergistic autoregulating combinations of cell surface LS and cell secreted LS [8]. Such combinations are represented by synbiotic LS (SLS as synergistic autoregulating combinations of PB surface LS and extracellular LSPB). LSPB participate in network recognition of mucin type glycoconjugates (MGC) possessing opposite relationships towards LSPB (for example, opposite relationships in registration of recognition of a set of LSPB of different taxonomic sources by a single MGC). LSPB are often used in combinations to Ab due to lectin ability to recognize intraantigenic structures, complete pictures of Ab binding, and realize high permeability into biogels [6]. LSPB induce cytokine production by human protective cells and systems. Probiotic/ useful LS can be used in synergistic combinations to enzymes and their modulators, other recognition molecules [6-8]. Preparations of acidic and alkaline LSPB are effective in anaerobic conditions (natural conditions in internal tracts and lumens of organism), do not contain oxidoreductase systems. Similarly to some phytolectins of mucin type (for example, mucopolysaccharide form of phytohaemagglutinin from kidney bean [PHA-M, mucoid form of PHA]), LSPB induce production of tumor necrosis factor-alpha by human peripheral blood cells, regulate migration of peritoneal macrophages in completing MGC influence manner, cofunction to other human protective cells and systems [8, 9].

In the presence of enzymes of limited hydrolysis, LSPB are become as sources of additional pools of probiotic effectors (oligopeptides- and glycopeptides-containing substances) of increased permeability. LSPB are members of new class of pathogen (as in cases of yeast like fungi [YLF] and staphylococci) biofilm destructors [10, 11]. LSPB

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reveal antimicrobial synergy to antibiotics and food phytolectins from grasses of medical significance [8, 10-12]. Autoregulated and other biotope systems involving "PB (cells)—LSPB" represent probiotic molecular-cellular biotope compartment against pathogenic compartment [13-15]. PB expressing and producing LS are characterized by capability for pathogen growth suppression, degradation and lysis of formed pathogen massive of YLF and Gram positive bacteria. Population pools of biotope PB alter distribution of microecological niches of pathogenic YLF species (*Candida albicans, C. tropicalis*) which can be become more available to antimicrobials. LSPB imitate main protective functions of PB [8] and possess preferential (compared to PB) useful properties for organism: they can be used non-dependently on biotope origin; they do not require special PB survival conditions in biotope (control of absence of wide set of antibiotics and other factors toxic for PB). LSPB initiate, direct, protect, conserve and correct assembling human cells which were attacked with system hydrolases (proteinases and glycosyl hydrolases) of surrounding as in case of biofilms possessing exposed antigens of mucosal cellular layer including dedifferentiatied in some extent tumor like cells possessing minimal décor can contain. Synbiotic LS (SLS: secreted and cell surface bound LSPB plus prebiotics) participate in "Microbiocenosis Quorum Sensing" (for example at the level of biotope autoregulating lactobacillar SLS which influence probiotic and pathogenic compartments [13-15]). SLS also influence biotope redistribution of YLF species influenced by probiotic like pools of strains from the same biotope [16] and "Cross-Talking to protective host systems" (peritoneal macrophage migration, blood lymphocytes production of cytokines, complement system C4B isotype as lectin like (which function in the presence of kidney bean phytohaemagglutinin [PHA] and wheat germ agglutinin [WGA] that are able to form specific complexes to MGC such as mucopolysaccharides and mucins) [8, 17, 18].

We developed approach of ranging/ ranking functional parameters of microbial probiotic strains to support constructed or predict new completed each other combinations of multistrain probiotics among lactobacilli and bifidobacteria [19-22]. Application of such rangings allowed establishment of biotope PB pools of strains and leader strains which are involved in redistribution of other biotope microbial species and subspecies populations [23-23b].

As part of human glycome, Ser/ Thr-glycans (O-glycans) of MGC play important role in supporting organism healthy balance, and mucins possessing established types of glycans reveal antipathogenic actions [24-26]. Microorganisms of mucosa influence mucosal architectures, and composition of microorganisms reflects functional status of mucosa (normal, pathologic or intermediate). Probiotic microorganisms (lactobacilli, bifidobacteria, others) interact to local mucins represented mucosal layer and mucosal cell patterns. Network of these relationships are broken in conditions of pathology (under action of pathogens, in cases of pathogen-induced tumor like cell degeneration) when PB are presented in very low amounts or absent (on the background of the presence of system characteristic pathological processes resulting in appearance of altered MGC antigenic structures) [27-30]. In spite of involving a set of human polymers in antimicrobial protection in human organism [31], LSPB oriented on MGC systems (MGCS) [32, 33] were not still taken into consideration in mucosal innate immunity. The aim was to propose concept of mucosa involving LSPB and MGCS in increasing organism resistance to pathogenic microbes and viruses.

2. Materials and Methods

Sources of proteins and LS were cultural fluids and cell surface of PB (Table 1). We used solubilized LSPB separated by sterile microfiltration, membrane ultrafiltration (fraction > 27 kD), precipitation variants, and isoelectrofocusing (IEF) in gradients of pH within interval 2-9 (pH 4-8, 3-5, 2-6) in slabs/ plates of PAG. LSPB were electroblotted on hydrophobic membrane or isolated from established lectin local parts of gel [6, 8, 9]. Multiple system forms of human recombinant protein hormone (commercial preparations of erythropoietin, rhEPO) separated by IEF-PAG followed by electroblotting on membrane were used as example of cytokine LS (CLS) [9]. MGC interacted to LSPB were established by MGC binding to blotted LS or to cell surface sensibilized with lectins. The following set of water soluble mucin imitating GC (artificial glycoconjugates with known chemical structures containing linear core PAA chain and side short branches of glycoside residues imitating cluster branches of mucin type glycans, www.lectinity.com; Table 2) was used: 1) L-Fuc-alpha-1-PAA, alpha-L-fucan-like, 2) D-Gal-beta-1-PAA, beta-D-galactan-like, 3) Gal(3-Sulfate)-beta-1-PAA, 3-HSO_3Gal-beta-1-PAA, 4) beta-D-galactan-3-Sulfate, 5) GaNAc-alpha-1-PAA, polymer containing poly(Tn-like antigen), 6) GalNAc-alpha-1,3-Gal-beta-1-PAA, A_{di}, poly(blood AII-group-like antigen)-containing polymer, 7) GalNAc-alpha-1,3-GalNAc-beta-1-PAA, F_s, poly(Forssman antigen-like)- containing polymer, 8) GalNAc-beta-1-PAA, asialylated mucin-like polymer, 9) Gal-beta-1,4GlcNAc-beta-1-PAA, poly(LacNAc)-containing mucin-like, 10) GlcNAc-beta-1-PAA, chitin-like soluble non-branched, 11) Man-alpha-1-PAA, alpha-D-mannan-like, 12) Man(6-phosphate)-alpha-1-PAA, 6-H_2PO_3Man-alpha-1-PAA, alpha-D-phosphomannan, 13) (MurNAc-L-Ala-D-isoGln)-beta-1-PAA, MDP-PAA, poly(muramylpeptide)-containing polymer, (bacterial peptidoglycan)-similar polymer, 14) Rha-alpha-1-PAA, alpha-L-rhamnan-like, 15) Neu5Ac-alpha-2-PAA, 16) Neu5Ac-alpha-2,3Gal1,4Glc-бета-1- PAA, 3'-SiaLac-PAA, 17) Gal-alpha-1,3GalNAc-alpha-1-PAA, poly(antigen $T_{\alpha\alpha}$-like)-containg polymer, 18) $CH_2(HOCH)_4CH_2NH$-PAA as control.

Interactions of MGC to LSPB or CLS were studied using: a) cell systems in micropanels in cases of haemagglutination (HA) or yeast agglutination or cytoagglutination inhibition (dissotiation of agglutinates in the presence of MGC added); in cases of biofilm forming and its dissociation, degradation and lysis; b) cell free procedures

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on electroblotted membrane. The following cell systems were used: a) human A(II) group red cells possessing available residues of GalNAc- (native and trypsin- or *Clostridium perfringens* sialidase-sensitized red cells), b) commercial baker yeast (*S. cerevisiae*) from local source (suspensions resistant in time were prepared and used) possessing exposed alpha-mannans and alpha-mannan-phosphates, c) YLF, lactobacilli, bifidobacteria and staphylococci from Collection of microorganisms of G.N. Gabrichevsky Research Institute.

Proteins were detected on membranes using fluorescent dye SYPRO Ruby Protein Blot Stain (Bio-Rad Lab) [36, 37]. LSPB separated forms on membranes were established by treatment of blots with GC-biotin followed by treatment with streptavidin-peroxidase conjugates. In addition, combinations of MGC and monoclonal Ab (mAb) against rhEPO were used for staining CLS in the following sequence: rhEPO—(primary mAb, IgG fraction)—(capture Ab against murine IgG)-biotin—Streptavidin-peroxidase. Treatments of blotted LS with MGC were before or after using mAb. For blot treatments, Twin 80 or Twin 20 (up to 0,1%) was used instead of 1-3% bovine serum albumin (in 10 mM phosphate saline buffer pH 7.2-7.4 at room temperature or at higher temperatures in special cases) [17]. Chemiluminescence of the bound peroxidase was detected in the presence of peroxidase chemiluminescent substrate.

Chemiluminescence or fluorescence was registered in optimal regime of real time in the Dark Room of BioChemi System (UVP, Calif.). Fluorescence exciting was at 254 or 365 nm. Fluorescence or chemiluminescence emission was registered using Bromide Ethidium or Coomassie filter. Sequential expositions were usually within interval (1 second) – (20 minutes) for chemiluminecence step-by-step accumulation [38-40] or within millisecond interval (1-5,000 msec) in case of fluorescence emission detection [36, 37]. Kits of proteins with known molecular masses and pI were used. Positions of proteins and major or minor components of LS on blot were calculated and ranged as relative intensities of components in linear gradient of pH (relative distant position of component or groups of components between cathode and anode were established).

3. Results and their discussion

3.1 Properties of probiotic proteins, LSPB and GC used

Some general properties of probiotic proteins, LSPB and GC used are shown in Tables 1 and 2.

3.1.1 Properties of probiotic proteins and LSPB

General properties of bifidobacterial and lactobacillar proteins. Some general properties of probiotic proteins, LSPB and GC used are shown in Table 1. Protein massives were as more acidic or more cationic for lactobacilli or bifidobacteria, respectively. Lactobacillar proteins revealed early lower and less prolonged fluorescence compared to bifidobacterial ones. Compared to maximal protein bands registration using Coomassie filter (exciting at 254 nm), the use of Bromide Ethidium filter (exciting at 365 nm) allowed obtaining highly discrete strain/ specie/ genus (protein blocks and/or groups)- specific patterns. The latter procedure allowed revealing/ screening pI-depended forming supramolecular multifunctional fluorescently active complexes possessing high potential of biorecognition and participation in energy transportation (different pathways for complexes of lactobacillar and bifidobacterial origin). In general, protein massives were characterized by (few protein block)-pattern distribution. Protein massives were preferentially localization within acidic intervals of pI. Protein patterns reveal strain/ species/ genus typing properties (the typing was impossible in Coomassie regime or was locally limited because of local undiscrete spot compactness of protein massive).

Proteins of lactobacillar PB. Lactobacilli revealed more discrete block patterns compared to bifidobacteria. Among ingredient strains of Acilact, protein massive of strain K_3III_{24} was characterized by maximal numbers of protein groups and their blocks. In addition, this strain produced significant amount of cationic low molecular bacteriocin like substances possessing increased detergent like properties. In gradient pH 4-8, lactobacillar proteins were represented by at least 29 major groups within 4 main blocks (in order of decreased fluorescent intensities): block-1(pI 4.4-5.37; 7 groups) > block-2(pI 5.37-6.10; 6 groups) > block-3(pI 6.15-6.9; 7 groups) > block-4(pI 7.0-8.0; 9 groups). Acilact was characterized by the presence of cationic peptides (strain K_3III_{24} - the main contributor).

Cell surface proteins of Acilact and its ingredient strains were individual and localized in region pI 4-6 (Acilact revealed more than 18 protein groups). Maximal differences between Acilact and strains were found in region pI 6-7. Oxidoreductase system pI 5.1-5.5 in Acilact was represented as oxydoreductase subsystems of ingradient strains (with exception of NK1) and *L. plantartum* 8RA3 (ingredient of Lactobacterin) in potential extended probiotic lactobacillar consortia including Acilact and Lactobacterin [2].

Proteins of bifidobacterial PB. Upon comparison of protein massives and LSPB in region pI 7.5-8.0, genus differences were revealed: characteristic sets of alkaline/ cationic proteins and LS for bifidobacteria compared to absence of such proteins and LS in cases of lactobacilli (bifidobacteria and lactobacilli completed each other). Bifidobacterial cationic proteins were resistant to hydrolysis (were protectd by local EPS). Cationic proteins and LS were used by us for strain typing.

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LSPB of strains studied. On example of industrial strains (Table 1), it is seen diversity of LSPB as well as their antipathogenic potential as ingredients of probiotics and biologically active additives (BAA). LSPB revealed strain/ specie/ genus- depended features. Among LSPB major and minor forms (major and minor subsystems of LS) were observed. Among LSPB of differents strains of lactobacilli and bifidobacteria, two or more lectin forms revealed similarity in specificities, and other forms were different (unique patterns of forms and ranging sets of forms in intensities as typing characteristics of LSPB were evaluated). Lectin components were able to recognize few types of GC (ranging set of GC intenscities served as characteristics of lectin form). The strain major components of LS may indicate strains as producers of major lectin subsystems (major lectins), and strain minor components of LS may indicate signals of lectin origin. In general, LSPB were represented as acidic (pI 3.5-4.5), less acidic (pI 4.5-7) and alkaline (pI > 7) components in cases of lactobacillar probiotic ingredient strains (*Lactobacillus* lectins [LL] 62-80 kD) and bifidobacterial probiotic ingredient strains (*Bifidobacterium* lectins [BL] 52-64 kD) [8]. LSPB contained cations of Ca^{2+} and Mg^{2+}. They also bound Li^{+} and Ru^{2+}. Ru^{2+} significantly amplified number of protein carriers of metal cations. The number of active recognizing components of LSPB was increased in the presence of detergent and detergent like agens [8]. Ability of less acidic lactobacillar LSPB to bind LacNAc-alpha-PAA was decreased in order: NK1 > 100_{ash} > Acilact. L-Fuc-PAA-binding LS (pI 3.7-4.3; block of 2-3groups of proteins which were colosed to pI 3.8-3.9) were more expressed in cases of lactobacilli (stronger and more pI-prolonged) compared to bifidobacteria. MDP-PAA-binding LS were similar in intensities for both lactobacilli and bifidobacteria. They were placed in protein massive pI 4.7-5.0

The establishment of strain different LSPB can help in choice of wishable strain combinations resulted in extending spectrum (or obtaining spectrum possessing increased few types of activities) of useful biological activities of combined LSPB when strains complete each other. Such approach adds principles of constructing probiotic consortia of human lactobacillar and bifidobacterial strains [20, 21].

3.1.2 Recognition properties of GC used

On example of MGC (Table 2), it is shown their universality and system action MGC in interaction to LSPB and CLS when strain/ specie/ genus types of LSPB—MGC assembling patterns on blots are observed [8, 31-33, 39, 40]. Our results indicate that MGC reveal useful/ probiotic properties and may act as imitators of probiotics. Together with probiotic LS, MGCS reflect synergistic power antimicrobial sets. Expected directions of actions of network complexes LSPB—MGC adapt further synergistic development of network cascades in directed networks of LSPB and MGC that support healthy biotope status. Such networks increase effectiveness of intercellular recognition (also taking into account synergy of direct and opposite complementary recognitions: [lectin of cell type 1]—[GC of cell type 2], [lectin of cell type 2]—[GC of cell type 1], etc.) and durability of intercellular assemblies of model human systems "First dedifferentiated cells—Prolonged survive of cellular massive". Our data support prospects of LSPB possessing distant antimicrobial activities against YLF and Gram positive pathogens.

3.2 Main states of concept of antipathogen LSPB—MGC supersystem

Aforementioned data on probiotic microcenosis LSPB and artificial MGC indicate important potential of their use against microbial pathogens as part of mucosal innate immunity in organism.

3.2.1 Main states of concept of immunity of organism involving LSPB—MGC supersystem of probiotic microbiocenosis

1. Principle of pore PAG organization involving model system LSPB-PAG and PAA linked MGC (MGC-PAA) are adequate to structure-functional organization of mucosa.
2. Mucosa is characterized by space architectures (of such contributors as host cells and microbiocenoses), programmed evolutionary and involutionary events (biorhythms as periodical elimination and exchange of mucosa; induced biosynthesis and degradation; others).
3. Major structures of mucosa are hierarchically ranged as Mucosa—(Mucosal upper and internal layers)—(Cell barrier)—(Epithelial cell surface)—(Membrane mucins). Regulation of this sequence of events in direct and opposite directions takes place.
4. Local mucosal phenotypes reflect biotope localized microbiocenosis, relationships between synbiotic and pathogenic compartments of biotope.
5. Sensitivity of YLF (eukaryotic communicative pathogens and eukaryotic microbial mediators of relationships between biotope bacteria and host systems) to LSPB serves as key indicator of mucosal anti-altered-human-cells' (anti-tumor-cell-like) potential. Early dedifferentiated host cells can serve as indicators of tumor processes [27].
6. Mucosa orders mucosal layer and cell barrier for localization, submission of surrounding, fixation and inactivation of pathogens (also together with complement system), for exclusion of pathogen distribution all over organism, for prevention of transformation of epithelium into tumor layer like.
7. It takes place coupled revealing antioxidant, antimicrobial, antiviral and antitumor activities of mucosa.

8. The chosen model LSPB— MGC supersystem is adequate to structure-functional organization of mucosa.
9. Recognition of mucosal ingredients in mucosal local surroundings by binding to molecular and cell surface targets are running by natural way involving LSPB, MGC and LSPB—MGC active complexes. These events influence structural organization of mucosa (pore network, availability of pores, types and levels of summary and local affinities).
10. LSPB (alone or in complexes to MGC) influence architectures of mucosa that simplifies delivery of MGC, their retention and further batched release (mucosa serves as adjuvant). Local detergents and detergent like factors increase number of LPSB antipathogenic derivatives. Local hydrolases (endogenic and /or delivered) involve in amplification of soluble signals of GC-recognition. As a result, antipathogenic pool of molecules is increased, and allergen factors of such pool are decreased.
11. LSPB control pathogen appearance within mucosa.
12. SLS initiate, stabilize, support and conserve biotope healthy status of microbiocenosis (as cells and cell associates separately bound to mucosa or as bound biofilms).
13. It is of rationality to use combinations of *Lactobacillus* LS together with *Bifidobacterium* LS [21, 22] because of lactobacilli stimulate and stabilize bifidobacteria in surrounding in addition to opposite regulation in direction *Bifidobacterium—Lactobacillus*).
14. Diversity of MGC provides latitude of adaptive mucosal replies to stress, increases therapeutic potential. The choice of MGC depends on mucosal biotope, individual available status and diagnose of patient.
15. Branched on duty network LSPB—MGC increases potential of mucosa against viral and other tumor inducers; supports mucosa as source of therapeutic MGCS together with other related effectors and cascade derivatives.
16. Mucosa and its microbiocenosis function as communicative societies - "bodies" at the level of supracellular (hierarchically higher than cells) signal exchange between mucosal body and microbiocenosis body involving LSPB and MGCS.
17. Mucosa serves as model library/ catalog of spectrum MGC, diagnostic indicator, sensor accumulator/ multiplier of "tumor cell like" antigenic signals.
18. SLS based mucosa is useful "device" for improved delivery, deposition and further release of therapeutic agents; for changing cell surface décor design. It can serve as the basis for successful using Ab against cancer antigens, other suppressors of pathogens, cell surface stabilizers.
19. Mucosa posseses adaptive multifunctionality and reveals the following network features: replies on stress (prevention of strong deviations in surrounding), functioning as traps (for example, of radicals and diagnostic antigens), local delivery, execution of adjuvant events, conversion of surrounding into stable state, adaptively dosed prolonged "vaccination" within surrounding, therapeutic action, openness to cofunctioning (for example, in communications such as Quorum Sensing and Cross-Talking), correction of processes of biorecognition/ local isolation and conservation/ prolongation in on duty regime by opposite relationships.

3.2.2 Strategies of application of mucosal LSPB—MGC superystem against pathogens

Aforementioned concept allows the following strategies of application of mucosal LSPB—MGC supersystem against pathogens.

1. *Support of biotope mucosa as naturally stable healthy one possessing microbiocenosis with increased resistance to pathogens*. Support is characterized by expressed active LSPB/ SLS. As a result of additionally delivered SLS into biotope, constructions "SLS—Mucosa" will increase mucosa-induced structure-function conservation and stability of healthy biotope, biotope resistance to negative or unwishable changes (for example, to appearance and amplification of pathogenic microbes and viruses as well as pathogen-induced early tumor like cells). Delivery of probiotic conbsortia and probiotic leader strains into biotope and support their survival.

2. *Regular delivery of SLS based constructed mucosa in problem biotope will allow regular exchanging altered mucosa* (for example, in case of mucosa saturated with bound MGC from surrounding) and allow reallocation of events to support healthy biotope status (for example, in cases of rectal and urogenital mucosal barriers).

3. *Delivery of constructions "(SLS—Chosen set of MGC)—Mucosa" will result in increasing not only antioxidant state of biotope but also allow using them as sources of addressed antimicrobial and antiviral preparations and vaccine ingredients based onMGCS.* Charged natural polymeric GC reveal antimicrobial and antiviral activities [41, 42]. Constructions "(SLS—Chosen set of MGC)—Mucosa" as supporting one will increase adapted properties of surrounding and target-depended effectiveness of delivered therapeutic agents (Ab, antigens, enzymes, antibiotics and bacteriophages) into biotope.

4. *Other strategies of mucosa using based on cell free LSPB against LS of pathogens (LSPB as concurents and antagonists of LS of pathogens).* Additional antimicrobial strategies of (cell free LSPB)-containing mucosa include the following main synergistic combinations of LL and BL: a) against pathogenic YLF (*C. albicans*, BL > LL) and pathogenic Gram positive bacteria (*S. aureus*, LL > BL); b) anti-*C. albicans* cascade "acidic BL—alkaline LL"; c) anti-

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C. albicans combinations of LSPB and grass lectins, BL and azoles. Advantages of such systems are in non-dependence on the presence of PB needed special conditions for survival.

5. *The use of natural antipathogen cofunctioning LSPB and biosurfactants and EPS.* Being in space region of EPS, LSPB will participate in distant multilayer assembling EPS in pore biogels as well as in distant antimicrobial control within such gels. EPS degradation with PB depolymerases will allow additional release of prebiotics, antioxidants and antitumor effectors into surrounding.

6. *In case of appearance of first tumor cell like partially dedifferentiated cells, SLS based mucosa will function in accordance to strategy of "reversible overbuilding or reversible rebuilded decoration" of altered cells when changed local mucosa will be overbuilt and get closer to major/ dominated original mucosal massive that will prevent further amplification of tumor similar cells and further tumor development.* It is simultaneously possible to increase tropism of altered cells to antitumor agents.

7. *Cofunctioning Ab-non-dependent network LSPB— MGC and human complement system involving complement component C4* [17, 18]. In addition, probiotic phytolectins(PHA and WGA) possessing a number of useful actions in organism cofunction to complement component C4B in binding some lipopolysaccharides of Gram negative bacteria [17, 18]. As a result, spectrum of directed synergistic probiotic and probiotic like agents (possessing different additional mechanisms of action) against pathogens is extended.

8. *The use of LSPB as universal inter- and intraregulators of enzymes* [6, 7]. LSPB against virulent factors including hydrolases (direct inhibition of hydrolases with LSPB, LSPB regulation of receptors containing enzymes in their masking—demasking; LSPB-enzyme complexes as antimicrobials; participation of LSPB type glycosyl hydrolases in forming prebiotics).

4. Conclusion

Results indicate importance of SLS, MGCS and SLS—MGCS in supporting antipathogenic capacity of any biotope mucus (intestinal, urogenital, others) as well as in increasing organism mucosal immunity. Mucosa possesses adaptive multifunctionality and reveals network of the following events: replies on stress, scavenger and trap reactions, delivery in and out, adjuvant retention and release, stabilization and prevention from unwishable changes, vaccination, therapy, prophylaxis, communications of Quorum Sensing and Cross-Talking types. These events correct processes of recognition/ isolation/ conservation/ prolongation in regime of preservation of on duty "return relationships". Concept and concept based strategies support balanced using SLS and LSPB in mucosa which is become more resistant to stress. Mucosa is become directly regulated multipotent affine architecture possessing distant control involving spectrum of chosen SLS—MGCS complexes. The strategies indicate prospects of development and application of mucosa in therapy of systemic and other infectious diseases. Concept and strategies are important for modeling and testing mucosa structural elements and functional ingredients. Results can be useful for further development of experimental approaches to biomedical engineering. SLS—MGCS supersystem serves as important part of innate antibody-non-depended protective system in organism which support and complete action of human complement system [43].

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Table 1 Antipathogenic potential of bacterial proteins and LSPB – ingredients of probiotics and BAA [2, 8, 20, 21, 36-40].

No	Probiotic strains* (previous names)	Proteins, LSPB	Probiotics and BAA based on ingredient strains [2, 34, 35]
1	*B. angulatum* OV15***	Unique protein massive among bifidobacterium tested. The absence of L-Fuc-PAA-binding LS. Man-alpha-PAA-binding LS (arounf pI 3.0) differing from No 11.	Prospects of use as ingredient of BAA.
2	*B. bifidum* No 1	Distribution of proteins similar to No 5 and 4; alkaline LS includes at least 3 protein groups associated to EPS.	Bifidok, Bifidumbacterin, Bifiliz, Normospectrum.
3	*B. bifidum* No 1 + *E.coli* M17	Acidic proteins >> alkaline proteins.	Bificol.
4	*B. bifidum* 791	L-Fuc-alpha-PAA-binding LS is around pI 3.9 within protein massive pI 4.0-4.4.	In consortium „*B.bifidum* 791+*B.longum* V379+ *B.breve* 79-119+*B.infantis* 73-15+*B.adolescentis* G7513”.
5	*B. bifidum var.* X	pI-Extended spectrum of alkaline LS expressed in high level.	Prospects of use as ingredient of BAA.
6	*B. breve* 23***	Unique protein massive among bifidobacteria tested; maximally high alkaline pI-proteins expressed.	Prospects of use as ingredient of BAA.
7	*B. gallinarum* GB****	High level of alkaline LS; dfferences of alkaline LS between race 3 and race 4 of strain in alkaline interval of pI.	BAA.
8	*B. infantis* 302-87***	Alkaline LS associated to EPS which is expressed higher compared to EPS of No 2.	Prospects of use as ingredient of BAA.
9	*B. longum* V379M	Major forms of Man-alpha-PAA-binding lectins with pI 4.5-4.6, GalNAc-beta-PAA-binding lectins with pI within 4.0-4.2 , L-Fuc-alpha-PAA-binding lectins with pI within 4.0-4.4.	In consortia with other probiotic bifidobacteria.
10	*B. longum spp. adolescentis* MS42 (*B. adolescentis* MS42)	At least 29 groups preferentially acidic proteins (> 27 kD) in interval pI 4-8; cationic proteins were represented > 21% of total protein forms (proteins with pI > 7.7 were masked with EPS; major protein forms are characterized with high intensities of chromophores.	Bifidin, Biovestin.
11	*B. pseudocatenulatum* OV2	Man-alpha-PAA-binding LS (up to 3 groups of proteins within pI 3.0-3.2) differing from No 1.	BAA.
12	*L. casei/paracasei* K_3III_{24} (*L. acidophilus* K_3III_{24}, *L.acidophilus* KAA)	Production of cationic peptides. Acidic LS is prepresented as Man-PAA-binding and includes components decreasing in order: pI 4.62> pI 4.02, pI 3.92, pI 3.95> pI 4.52.	Acilact, Normospectrum.
13	*L. helveticus* 100_{ash} (*L. acidophilus* 100_{ash}, *L.acidophilus* JCH)	Minimal protein massive (as discrete numbers of forms) among ingredient strains of Acilact.	Acilact.
14	*L. helveticus* NK1 (*L. acidophilus* NK1, *L.acidophilus* NKJC)	8 protein groups decreased in order: (59-61 kD)> (200-250 kD)> (27-28 kD)> (49-51 kD)> (34-36 kD)> (18-20 kD)> (14-16 kD)> (11-13 kD). The absence of oxidoreductase system pI 5.3-5.7. Pattern of protein distribution which is similar to Acilact. Acidic LS are preferentially 38-80 kD, pI 4.2-5.2. Man-alpha-PAA-binding LS is mainly within pI 4.6-4.8; GalNAc-beta-PAA-binding LS is characterized with pI around 4.2 within region pI 4.0-4.4 (lower compared to bifidobacteria), L-Fuc-alpha-PAA-binding LS is within pI 4.0-4.4 (plus additional lower region within pI 3.6-3.7); the presence of low expressed MDP-PAA-binding and A_{di}-PAA-binding LS.	Acilact, Acipol, Hilac-Forte, Normospectrum, Polybacterin.
15	NK1 + K_3III_{24} + 100_{ash} (Acilact)	Increased potential of cationic peptides compared to ingredient strains. Distribution of proteins similar to NK1. In addition to own unique components of LS, LS contain components of LS similar to No 14, 15.	Acilact.
16	*L. paracasei* VKPM V6253** (*L. amylovorus* VT24/88)	High level of LS (> No 15).	Lactoamylovorin.
17	*L. plantarum* 8RA3	Maximal level of Man-PAA-binding LS compared to ingredient strains of Acilact.	Florin-Forte, Lactobacterin, Polybacterin.
18	*S. thermophilus spp. lactis*	Massive of acidic proteins similar to No 14.	Danacor.

Comments: *from collection of microorganisms of G.N. Gabrichevsky Research Institute for Epidemiology & Microbiology **from breast infants; ***from hog cecum; ****from chicken cecum; others – from human gut. LS separated by isoelectric focusing in PAG in gradients pf pH within

interval 2-9, followed by electroblotting on membrane and treatment with GC-PAA-biotin—Streptavidin-peroxidase. Proteins on blot were detected with SYPRO blot stain (BioRad Lab.). EPS= exopolymeric substances.

Table 2 Recognition activities of GC used.

No	Glycoconjugates (without spacer, PAA and biotinyl label)	GC-modulation of LS-induced bioprocesses, comments
1	**L-Fuc-alpha-1-	Strong visualization of CLS (also in combination with mAb to rhEPO) on blot (strong overloping to No 9); pI-prolonged pattern compared to that in case of No 9; especially in cases of genus *Bifidobacterium*; binding to lactobacillar acidic LS depending on *Lactobacillus* specie strain.
2	Gal-beta-1-	Diffuse patterns of LL on the blot, stabilization/ activation of aLSPB-induced HA (partial elimination of cytoagglutinate-dissolved activity); binding to CLS on the blot is lower compared to No 9.
3	3-HSO_3Gal-beta-1-	Preferential binding on the blot to major forms of acidic LSPB (stronger binding in comparison to No 12).
4	GaNAc-alpha-1-	Maximal HA inhibition of LSPB compared to No 11, 12; low binding to LSPB on the blot.
5	A_{di}-	Strong visualization of LSPB on blot; specific pattern in addition to characteristic patterns in cases of No 1, 6 and 8; preference in monitoring LSPB (increased sensitivity, decreased exposition time) in comparison to No 8; strain- and specie-type dependence of simultaneous binding to limited number of major and minor forms of LS on the blot; more compact blot LSPB pattern compared to the pattern in case of No 6 (distinct from other GalNAc-containing simple antigens).
6	F_s-	Strong visualization of aLSPB on blot; complement of patterns in cases of No 5 and 8; preference for monitoring LSPB in comparison to No 8; more discrete and more pI-prolonged patterns of LSPB on the blot in comparison to No 5 (recognizing GalNAc- containing simple antigens by system LL and LB).
7	GalNAc-alpha-1,3GalNAc-alpha-1-	Low binding to LS on the blot.
8	GalNAc-beta-1-	Low binding to LS on blot (some less time before beginning chemiluminescence emission of labeled protein complexes on the blot in comparison to No 4); maximal number of CLS forms on the blot (in combination with mAb).
9	LacNAc-	Strong visualization of CLS on the blot (also in addition to pattern in case of using mAb) (strong overlapping to pattern in case of No 1); more compact blot CLS pattern compared to the pattern in case of No 1.
10	GlcNAc-beta-1-	Low similarity to pattern in case of No11 in reaction of HA inhibition of LSPB.
11	Man-alpha-1-	High inhibition of aLSPB-induced HA (differences between patterns of LL and LB) which can be increased by addition of No 12; inhibition of aLSPB induced yeast agglutination; similarity to pattern of aLSPB on the blot in case of No12.
12	6-H_2PO_3Man-alpha-1-	Similarity of LSPB patterns to patterns in case of No 11.
13	MDP-	Strain/ specie-type dependence of simultaneous binding to limited number of major and minor forms of LSPB on the blot.
14	L-Rha-alpha-1-	Binding to LS in external layer on the blot.
15	Neu5Ac-alpha-2-	Very low binding to aggregated forms of CLS on blot (it takes prolonged incubations for revealing the beginning chemiluminescence of labeled protein complexes on blot); quantitative differences in comparison to patterns in case of 16.
16	3'-SiaLac-	Low binding to aggregated forms of CLS on the blot (it takes prolonged incubations for revealing the beginning chemiluminescence of labeled protein complexes on the blot); quantitative differences in comparison to patterns in case of 15.
17	$T_{\alpha\alpha}$-	Without binding to bifidobacterial and lactobacillar LS on the blot.
18	$CH_2(HOCH)_4CH_2NH$- (glyciritol-)	Without discrete specific band patterns of binding to LS on the blot.

Comments: *Abbreviations are given along the text body; **L-configuration of sugar if it is not indicated.