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GLYCOCONJUGATES-BINDING PROTEINS, GLYCOCONJUGATES FOR BIOTECHNOLOGICAL AND BIOMEDICAL USING. AN OVERVIEW OF OUR PUBLICATIONS

Lakhtin V. M.*, Lakhtin M. V., Aleshkin V. A. and Kombarova S. Y.

G.N. Gabrichevsky Reseach Institute for Epidemiology and Microbiology, Moscow 125212, Russia.

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***Corresponding Author**
Lakhtin V. M.
G.N. Gabrichevsky Reseach Institute for Epidemiology and Microbiology, Moscow 125212, Russia.

ABSTRACT

Our results of biotechnological aspects of the study of glycoconjugates (GC) and GC-binding proteins are briefly summarized: diagnostic and prognostic imaging and typing of systemic components of the C4 complement component of patients, including subisotyping; desialylation of GC on the example of glycoproteins; determination of soluble and immobilized biosurfactants and their pattern visualization on the example of culture fluids (CF) of *Gram*-positive bacteria (GPB),; determination of (glyco)proteins, including the determination of hydrophobic proteins and peptides of CF of GPB; determination of fluorophores in CF of GPB; preliminary analysis of the proteome of CF of GPB by mapping CF components separated by electrophoresis; determination of protease systems in CF of GPB on the example of probiotic strains of lactobacilli; blot block analysis of functionally active cytokines on the example of (glyco)hormone - erythropoietin; the use of horseradish peroxidase chemiluminescence, including the acetate-binding substrate of the catalytic center, in the study of systemic GC; determination of the blot block series of oxidoreductases in phyto- and microbial biological preparations of various degrees of purification, including biopreparations of medical importance; blot block determination of lectin and lectin-like proteins using synthetic polymer GC (www.lectinity.com); micropanel and blot block analysis of functionally active isotypes C4A and C4B and their subisotypes; system analysis of the relationship of microbial cell and metabolite antagonists in biofilms with or without participation of antibiotics.

KEYWORDS: Glycoconjugates; glycoconjugates-binding proteins; gel electrophoresis; isoelectrofocusing; electroblotting; microanalysis; fluorescence; chemiluminescence; biological activities; functional activity of proteins; bacterial biosurfactants; systemic analysis of proteins; human complement system; protein typing of diseases; proteins of *Gram*-positive bacteria; probiotic metabolites; phytopreparations of medical importance; proteases; oxidoreductases; recognition; biofilms; lactobacilli; bifidobacteria; probiotics; biomedicine; biotechnology.

ABBREVIATIONS

AB	antibodies	EPO	erythropoietin
BF	biofilms	EPS	exopolysaccharides, exopolymers
BFF	biofilm forming	FL	fluorescence
BS	biosurfactants	GC	glycoconjugates
BSA	bovine serum albumin	GP	glycoproteins
CA	cytoagglutination	GP	glycoproteins
CDA	clot dissolved activities	GPB	Gram positive bacteria
CF	cultural fluids	HA	hemagglutination
CL	chemiluminescence	HCS	human complement system
ConA	concanavalin A	IEF	isoelectrofocusing
CSLF	cell suspension lectin forms	LS	lectin systems
EC	erythrocytes	PA	polyacrylamide
ehEPO	endogenic human EPO	PAG	polyacrylamide gel
EP	electrophoresis	PBS	phosphate buffer saline, pH 7
		rhEPO	recombinant human EPO

1. INTRODUCTION

The aim is to summarize our results on biotechnologies (methods, approaches, methodology), mainly in the study of the components of innate and probiotic immunity, involving glycoconjugates (GC) and GC-recognizing and binding molecular systems. The results are presented in several accented scientific areas.

2. RESULTS AND THEIR DISCUSSION

2.1. Determination of proteins and natural GC

Determination of proteins and (poly,oligo)peptide-containing GC is a routine daily laboratory procedure. At the same time, spectrophotometric methods are most often used,

and their choice depends on the object of study. The analysis methods based on fluorescence (FL) and chemiluminescence (CL) are comparable to radioisotope methods in sensitivity, but are relatively simple, safe and are not limited by special safety requirements for conducting an experiment.

The purpose is to summarize our data on the laboratory determination of proteins and protein GC: in solution and on surfaces, isolated and in culture fluids (CF), before and after electrophoretic separation, with registration of peptide bonds and aromatic amino acids available to external agents, according to proprietary and acquired FL, using CL sorbed oxidase; synthetic polymer soluble GC, enzymes and other functionally active agents and reaction cascades; the combined options listed above.

The modified/developed methods and methodologies, their application and prospects are described and discussed below.

2.1.1. Determination of hydrophobic proteins and peptides of CF of Gram-positive bacteria (GPB)[1,2]

The problem: the dependence of protein determination on the emulsification of CF (especially against the background of the severity of protein hydrolysis products), the choice of method. We have developed a comprehensive protein assessment without the influence of low molecular weight impurities and emulsification of CF, taking into account peptide bonds and additional control of the presence of a hydrophobic control protein - bovine serum albumin (BSA).

To evaluate the CF protein in solutions, the *Waddel* method for determining glycoproteins by the presence of peptide bonds was used: [µg/ml]= $D_{215} – D_{225}$) x 144, where D is the optical density at 215 and 225 nm; 144 is the coefficient calculated for BSA. The sensitivity of the method is up to 30 times higher than when determining the protein in the absorption peaks of Trp and Tyr. *Lactobacillus* cultures grown in CY-5s medium (casein-yeast medium with salts) in the stationary phase were used. The cream layer was removed after freezing the CF supernatant (by cutting off the top layer in a test tube), a concentrate without amino acids and peptides was obtained by membrane microfiltration/ sterilization of the supernatants through *Steriflip* (Millipore), followed by concentration (40-60 fold; to a residual final volume of 100-150 µl) and "washing" the concentrate by a phosphate buffer saline (PBS) in a centrifuge in a glass of *Centricon Plus-20* (Millipore):

hydrolyzed protein and colored impurities of less than 27 kD are removed. The sample volumes of the concentrates were restored to the original volume of the supernatant and the protein was determined.

Results

Complete removal of cream or boiling of the CF supernatant improved protein yield and reproducibility of its detection. Under refolding conditions (5-10 micrograms of protein/ml, PBS), there was a better convergence of the estimation of concentrations of BSA or IgG (in solutions of protein in PBS prepared by suspension) with the *Waddel* method. When using other methods of optical protein evaluation, the results of protein determination by the *Lowry* method were closer to those by the *Waddel* method than by the *Warburg*, *Christian* method [µg/ml]= ($1.45D_{280} - 0.74D_{260}$) x Dilution. *Acylact* and strain K_3III_{24} (but not 100_{ash}, NK_1) gave maximum protein levels. The method is convenient for comparative (and screening) protein analysis of a set of strains. The method is suitable for the determination of protein with a reduced content of Tyr and Trp. The method is not applicable for CF of Gram negative bacteria with stable opaque emulsions of lipopolysaccharides and high sorption on glass. Dot-blotting makes it possible to evaluate a pool of hydrophobic (BSA type) non-hydrolyzed proteins sorbed onto a hydrophobic membrane. The sensitivity of the method is 1 µg/ml.

Dot-blot fluorescent determination of the supernatant protein and its concentrate was carried out in parallel (in comparison with the registration of BSA). A series of dilutions of samples were applied to *Immobillon P* (Millipore), proteins were evaluated by their own (intrinsic) PH and after treatment with a fluorescent dye *SYPRO Ruby protein blot stain* (Bio-Rad) based on ruthenium (Ru^+) (sensitivity is comparable to colloidal gold and higher than that of silver nitrate, www.probes.com/syprodyes). FL was excited by ultraviolet light and recorded in the *BioChemi System* (UVP, Calif.). *Lactobacillus* supernatants contained up to 2 mg/ml of hydrophobic proteins (higher protein content values in the supernatants according to method-1 (modified "*Waddel*") were due to the contribution of the protein fragments present in the case of their limited proteolysis, as a result of which the number of protein molecules formally increases, as it could be in the case of strain K_3III_{24}). A coincidence of the protein determination in solution and on the blot was achieved. The use of both methods also makes it possible to assess the presence of peptides in CF and to judge the presence and effectiveness of hydrolases. The

sensitivity of the method is 1 µg/ml. *Prospects* of the method: rapid assessment of protein adhesion systems.

2.1.2. Determination of fluorophores in the CF of GPB[3-5]

The presence of fluorophores in solutions of proteins and GC indicates their potential in biorecognition, contributes to their highly sensitive detection.

Results

The possibilities of determining proteins and their complexes by the FL of aromatic amino acids and the shift of the FL maxima, respectively, are demonstrated. Low molecular weight fluorophores of CF supernatants in ordinal dilutions can be reliably visually detected by dot-blot analysis.

Prospects: further use of spectral analysis of protein-containing GC (combinations of absorption derivative spectra, fluorescent, circular dichroism spectra [CD spectra], atomic adsorption, mass spectrometric, in complexes with probes, others); evaluation of modified aromatic residues Tyr and Trp (sulfated, phosphorylated, others) in proteins; design of synergistic agents, their complexes, combinations with other effectors.

2.1.3. Analysis of the proteome of GPB in CF[6,7]

Electrophoretic evaluation of the protein in the CF supernatants of lactobacilli and bifidobacteria was carried out using two variants of a fluorescent dye SYPRO (for using within gel and on the blot).

The analysis of reconstructed (achieving the maximum number of bands compared by computer editing) FL protein patterns on blots with charge-separated proteins of the CF supernatant concentrate after IEF-PAG in the plate in the presence of urea and sucrose revealed up to 30-40 major bands ranked by relative content. In the case of separated groups of proteins by molecular weight (EP-PAG in the *Lemmli* system), a 1 msec exposure change (the sensitivity limit of the *Biochemi System* device variant used) made it possible to consistently accurately localize each protein band in the initial diffuse array in the PAG plate as highly discrete (bands as lines) with an accurate molecular weight, and to rank the severity of the bands by the relative content of CF in the concentrate / supernatant. The proposed methods of reconstruction of visual patterns are important for

screening and standardization of strains as well for diagnostic, prognostic and functional assessment (see below) of protein effectors.

2.1.4. Determination of protease systems in CF of GPB[8]

The analysis of the reconstructed FL patterns of proteins on the blot (patterns of protein blocks I [most acidic], II [less acidic] and IV [cationic]) allowed to judge the features of proteases (including caseinases and "caseinase+peptidase" systems) in the CF of lactobacilli (including the multi-strain probiotic *Acylact*) and bifidobacteria. The methodology is promising for the development of highly sensitive glycoproteins (GP)-specific visual pattern analyses of hydrolases.

1.5. Pattern block analysis of functionally active glycohormone erythropoietin (EPO)[9-13]

Dot-blot analysis

The *problem*: the lack of a simple, qualitative method for detecting recombinant human EPO (rhEPO) in the urine of a patient receiving therapeutic injections of the drug. Tasks to be solved: distinguishing endogenous human EPO (ehEPO) and rhEPO in biological fluid; speed of analysis (within two working days); simplicity (presence of urine concentrate, membrane blot and FL detector). *Principles of analysis*. As a rule, rfEPO used for therapy contain forms less acidic than ehEPO and can be removed under pH3.9 blot treatment conditions. Additional pH2.9 treatment also allows the removal of ehEPO. Heat treatments of blots-pH7 and blots-pH3.9 enhance the differences between urine preparations without or with rhEPO. The residual CL on the blots can be compared as part of a single combined blot under identical conditions.

Description of the method. Urine concentrates without and with rhEPO were used (concentration factor – up to 500 fold). A series of pillboxes (3-5 ml each) were created with dilutions of both concentrate preparations. EPO on blots was manifested by an immune sandwich in combination with biotin-streptavidin peroxidase. Sequential two-stage pH-treatment of the blots was performed (at pH 3.9 and then at pH 2.9) and then thermal treatment at 50-60°C. Residual CL patterns were recorded in the *Dark Room* of the *BioChemi System* (UVP, Calif.).

Results: working dilutions of concentrates were established, unambiguously indicating the presence of rhEPO in urine.

Prospects of the method: monitoring of the injected rhEPO excretion from the body of patients (with renal insufficiency, anemia, insufficiency of organic iron [hemoglobin and other heme or hemin-containing compounds]) of various types of commercial rhEPO; use instead of a commercial micropanel kit; application for the evaluation of recombinant interferons (similar to EPO on many physoco-chemical properties); use in immunochemical dot-blot assays based on CL of sorbed horseradish peroxidase.

The IEF-PAG followed by blotting of the separated systemic EPO forms in urine made it possible to detail the prognostic and diagnostic patterns of CL of forms of ehEPO and rhEPO and quantify the physico-chemical interconversion of ehEPO and rhEPO. A significant increase in the sensitivity of registration and discreteness of systemic forms of EPO was observed with: thermo (50-60^{o}C) washing treatments of blots, pH 8.5 (or more)-treatment of the blot before adding the substrate, in case of manifestation by an immune sandwich with registration of CL on blot at 55^{o}C. *Prospects* of the method: monitoring the increase in the resultant activity of ehEPO in the process of selected modes of administration into the body. The *sensitivity* of the method is the detection of EPO in the patient's urine 96 hours after a single injection of *Erythrostim* (Russian bacterial preparation of rhEPO).

2.1.6. The use of horseradish peroxidase CL in the study of systemic GC[14]

included all cases of the blot-bound enzyme: analysis of C4A and C4B isotypes using polyclonal antibodies (AB) to C4 labeled with peroxidase; manifestation of EPO by biotinylated immune sandwich followed by streptavidin peroxidase treatment; manifestation of lectins by peroxidase registration. CL was recorded in the *BioChemi System* (UVP, Calif., USA) as a series of patterns in the process of successive non-linearly selected time accumulations of a differentiated signal, when at the next exposure the signal starts from zero, passes through the maximum and reaches the background. The pattern with the achieved absolute maximum was selected. A chemiluminescent peroxidase substrate *BioWest* (Pierce Chemical Co., USA) with increased sensitivity and stability was used (it does not lose its properties in the form of a prepared working solution during the day at room temperature and daylight).

Results

1. Under the conditions we have chosen, the substrate consumption can be reduced by an order of magnitude. *BioWest* generates a signal 3.5 times higher than the ECL

substrate and 14 times higher than the *ECL Plus* substrate. *BioWest*, in the modes we selected, developed a higher CL intensity for a shorter exposure time of a blot with a sorbed peroxidase conjugate compared not only with ECL, but also "*SuperSignal ELISA Pico Chemiluminescent Substrate*" (Pierce Chemical Co., USA).

2. The use of an acetate-binding subsite in the catalytic center of horseradish peroxidase in the pattern block technologies for purification, separation and identification of C4A and C4B isotypes and their subisotypes made it possible to significantly improve the background (eliminate pH dependence, make it homogeneous).
3. An additional increase in the sensitivity of registration of the CL systemic forms of EPO manifested by the immune sandwich and biotin-streptavidin-peroxidase combination was achieved (see above).

2.1.7. Determination of the combined series of phyto- and bacterial oxidoreductases in biological products,

including those of medical importance.[15-17]

The objects were high-molecular salt (more than 27 kD) sterile extracts (with a low protein content) of phytochemicals from medicinal plants (dried and ground herbs of lapchatka (*Potentilla absinthifolia*), asterisks (*Stellaria ssp*.), Ivan tea (*Chamaenerion angustifolium* L. Scop.), and others) – ingredients of dietary supplements.

Results

The procedure IEF-PAG in the presence of urea and sucrose, blotting on Immobillon-P and subsequent manifestation of oxidases in the presence of chemiluminescent substrate *BioWest* revealed a serial (up to 5 bands) distribution of highly acidic (pI 3-4) aggregated oxidases dependent on plant species. Pattern blocks of slightly acidic oxidoreductases (pI 5-6) in biologics of probiotic strains of lactobacilli were detected.

Prospects of the method: the possibility of analyzing oxidases (including those with antimicrobial activity) in biologics of various degrees of purification with simultaneous detection of lectins by additional blot treatment using GC (systems GC-biotin—Streptavidin-peroxidase or [anti GC Ig-peroxidase or peroxidase immune sandwich]).

2.1.8. Pattern block determination of lectins and lectin-like proteins using synthetic polymer GC (www.lectinity.com)[18-23]

The determination of proteins as lectins was carried out using biotinylated pseudopolysaccharide and antigenic GC based on linear chain of polyacrylamide (PA) as side glycans, synonyms in parentheses: Fucα1-PA [α-L-fucan-like]; Galß1-PA [β-D-galactan-like]; Gal(3-Sulfate)β1-PA [3-HSO_3Galß1-PA; β-D-galactan-3-sulfate polymer]; GaNAcα1-PA [containing poly-(Tn-like antigen) polymer]; GalNAcα1,3Galß1-PA [A_{di} as poly(AII-blood group-like antigen)-containing polymer]; GalNAcα1,3GalNAcβ1-PA [Fs as poly(Forsman-like antigen)-containing polymer]; GalNAcα1,3GalNAcα1-PAA ; Gaα1,3GalNAcα1-PA [poly(Taα-like antigen)- containing polymer]; GalNAcß1-PAA [desialyzed mucin-like]; Galß1,4GlcNAcß1-PAA [poly-LacNAc-containing mucin-like]; GlcNAcß1-PAA [chitin-like soluble unbranched]; Mana1-PA [α-D-mannan-like]; Man(6-phosphate)α1-PA [6-H_2PO_3Manα1-polymer; α-D-phosphomannan]; (MurNAc-L-Ala-D-isoGln)β1-PA [MDP-PA; poly(muramyldipeptide)-containing a polymer; (bacterial peptidoglycan)-like]; Rhaα1-PA [α-L-ramnan-like]; glucitol-PA [as a control]. The listed water-soluble GC (0.5-5.0 micrograms/ml, in PBS) contained exposed short carbohydrate antennas repeatedly repeated in the form of random clusters (mainly from one or two carbohydrate residues) departing from the PA chain (www.lectinity.com). Concentrates of CF supernatants, protein fractions of various degrees of purification were used for dot-block analysis as well as pattern block analysis of separated IEF-PAG systemic forms of lectins. Biotinylated GC bound to proteins on the blot were treated with streptavidin-peroxidase, a chemiluminescent substrate was added, and CL was recorded in a live image in the *BioChemi System* (UVP, Calif., USA).

Pattern block analysis of the lectin systems of CF of GPB [20, 21]. Methods have been developed for the determination of drugs in the CF of lactobacilli and bifidobacteria (an expanded number of strains) by obtaining GC-dependent kinetic patterns of CL on a blot (before and after IEF-PAG). Types of drugs have been identified, depending on the types of GC (including those resistant to recognition by some types of drugs, interacting with antigens, recognizing GalNAc-containing GC antigens and pseudopolysaccharides); layered, with a maximum and minimum number of forms, genus/species/ strain-typed.

Determination of CF oxidoreductases of GPB.[17] The ability of oxidoreductases to selectively and reproducibly "discolor" FL and CL of electrophoretically separated and colored protein bands on the blot was used.

Results

The same block of bands (with a molecular weight of more than 27 kD) stained with SYPRO fluorescent dye in the pI 5 region (up to 5 highly discrete bands associated with Ru^{+}) of the ingredient strains of lactobacillar *Acylact* "faded" in a strain-dependent manner. A similar CL fading was observed and reproduced with repeated use (reprobing) of the blot for subsequent protein staining by the "GC-biotin—Streptavidin-peroxidase" system. The maximum multicomponent (in terms of composition within pI-interval) discoloration system was revealed in the case of a high-molecular-weight concentrate of CF of *Acylact*, which includes systems of ingredient strain different components (strain NK_1 is not a contributor to "fading"). The results are confirmed by the literature on the presence of dye-bleaching lactobacillar oxidoreductases, the presence of several lactobacillar oxidoreductases within the pI 5-6 region, as well as the existence of bifunctional "primitive" lactobacillar oxidoreductases of the type of nonhemin catalases-peroxidases.

Pattern blot block analysis of EPO as a lectin and a lectin system[21-24]

The problem: the search for new promising properties of therapeutic proteins, including their multiple forms. *Method.* CL analysis of systemic forms of rhEPO and ehEPO on a blot was performed after IEF-PAG in the presence of 7 M urea and 5% sucrose. EPO bands were manifested by a combination of two staining methods: immune sandwich and biotin-streptavidin-peroxidase system, GC-biotin-streptavidin-peroxidase treatment (and a combination in reverse order).

Results

The presence of multiple forms (to varying degrees) of independent binding sites of monoclonal antibodies (AB) (to an oligopeptide in the region of the N-terminus of the amino acid sequence) and a limited set of GC types was revealed. The use of a set of key GC (diagnostically significant, significant for drug identification) increased the number of detectable forms of ehEPO and rhEPO, increased the differences between commercial rhEPO preparations on the blot, including in areas with low affinity for AB. GC showed unequal protective (masking and antioxidant) properties in relation to active co-functioning supramolecular assemblies based on systemic forms of EPO.

The *prospects* of the method: screening of the most resistant as well as most sensitive forms of EPO in biological fluids in the presence of GC (special sets of GC), including under conditions of assemblies simulating events on the cell surface.

2.1.9. Micropanel analysis of functionally active C4A and C4B isotypes of the C4 component of the HSC[25]

Micropanel analyses of the functional activity of C4A isotypes (on sorbed aggregated IgG3), C4B (on sorbed Pyrogenal – lipopolysaccharide of *Salmonella typhi,* Scientific Research Center of Epidemiology and Microbiology named after academician N.F. Gamalei) and both isotypes in a hybrid micropanel (combining strips with IgG3 and Pyrogenal in wells; the advantage is the simultaneous in time identical multi–step determination of the ratio of isotypes from the same serum). After adding the serums and completing the assembly at the bottom of the wells, the reaction cascades were stopped, AB (to C4) labeled with peroxidase and the substrate were added. The color staining in the wells was recorded using a standard reader. Micropanel variants of the analysis of sera with pre-"intercepted" assembly activity of C4-isotypes of HSC with phytolectins and other GP were also proposed.

An independent confirming micropanel determination of the activity of isotypes is possible according to the data of the FL patterns of isotypes on the blot (see above).

Conclusion. The laboratory technologies described above and their components are available for widespread use. Blotting and micropanel methods are close to biochip technologies and this is their perspective. The possibilities of functional visual pattern microanalysis of enzymes and cascade constituents on blots are promising. The definitions of proteins and GC (see also other sections of the review) expand the possibilities of analysis. The proposed methodologies organize the methods in the sequence of their use, represent qualitatively new biotechnological products for microanalysis.

2.2. Determination of soluble and immobilized biosurfactants and their pattern visualization using the example of CF of GPB

High interest in the study of GC – GP, proteoglycans, biosurfactants (BS), exopolysaccharides/exopolymer substances (EPS), glycolipids, lipopolysaccharides is due to their active participation in the functioning of the glycome network in the body [26]. BS and EPS of GPB are promising multifunctional agents with antimicrobial, prebiotic, detergent-like, emulsifying and other biological effects. Fundamentals in the study of GC are the

development of methods and methodologies, the development of algorithms based on them, working hypotheses and concepts that stimulate further research. However, screening methods for BS and EPS, including using IEF—blotting analysis of human probiotic bacteria, are complicated, inaccessible or uninformative.

The *aim* is to summarize our progress in the field of methodology for studying BS and EPS.

Materials and Methods. The objects were soluble and immobilized BS and EPS of CF of probiotic strains of bifidobacteria and lactobacilli, including those isolated from the human intestine. CF supernatants and their high molecular weight (more than 27 kD) concentrates (concentration degree – 50-100 fold) obtained by membrane microfiltration through *Steriflip* (Millipore) followed by membrane ultrafiltration of the supernatants through *Centricon Plus-20* (Millipore) were used. The analyses of BS and EPS were carried out using methods developed by us (see below) in combination with highly sensitive kinetic detection based on FL, as well as using IEF separation of samples in the PAG plate in a pH gradient of 4-8 in the presence of urea and sucrose and using EP in the PAG plate with detergent in the *Lemmly* system, electroblotting on the membrane. Analysis of GC bound to a fluorescent dye *SYPRO Ruby protein blot stain* (Bio-Rad) or *SYPRO Ruby protein gel stain* (Bio-Rad) was after excitation of the blot with light at 254 or 365 nm, followed by registration of FL after a *Bromide Ethidium light filter* (*Coomassie light filter* was used as a control) in the system *BioChemi System* (UVP, Calif., USA).

The determination of soluble BS[27,28] was carried out using the method developed by us. A test system was pre-prepared: 30 µl of mineral oil (there is no effect in cases of sunflower, olive, or other vegetable oil, which makes it possible to study BS in conditions of bacterial growth on media with vegetable fat additives; the addition of excess mineral oil caused the "collapse" of oil-free lumen circles on the surface of the water into curved "double-layered sticks and ellipses" extended in diameter), 40 ml of distilled water was added to the surface in a glass crystallizer with a diameter of 11 cm. Samples were introduced with a polystyrene tip (a 50 µl tip was used) of 10 or 2 µl (a 5 µl tip was used). BS activity was determined by the average diameter of the enlightenment circles in a solid oil film on water after BS application. The background (minimal residual BS activity) varied in the range of 0.5-0.8 cm.

Results

When reducing the concentrate with saline to the initial volume of the supernatant or with further dilution of concentrates with saline by 100-10000 times, the activity of BS was completely lost, which indicated: a) a special state of activity of BS of high-molecular associates of concentrates of supernatants of CF, b) sensitivity of BS to the ionic force of the environment. The BS in the concentrates were stable during storage for more than a year at -35^{o}C.

The detection of BS was optimal in a container with a diameter of 10-11 cm with 40 ml of water and 30 µl of mineral oil (but not 40-50 µl – aggression of oil intp the circle, understated diameters); mineral oil is needed (petroleum oil as a "lighter", but not sunflower or olive oil - not spreading over the surface of the water, but remaining in the form of a three-dimensional consistency). BS were detected in concentrates (2-10 µl of samples per oil surface) and were absent in the ultrafiltrates of the supernatants. Ranking of BS activity (minus the background, the multiplicity of the difference and the decrease in the maximum value of BS activity as a percentage are indicated in parentheses when applying an excessive or minimum volume of concentrate [10 or 2 µl; 5-fold difference]): *B. bifidum* №1 (5,35-2,15; 2,49; 60%); *B. gallinarum* GB (4,55-2,65; 1,75; 42%); *L. casei* K_3III_{24} (3,55-2,05; 1,73; 42%); *Acylact* [K_3III_{24} +100_{ash}+NK_1] (3,35-2,15; 1,56; 36%); *L. helveticus* 100 % (2,35-1,75; 1,34; 26%); *B. longum* MC-42 (2,15-1,85; 1,16; 14%). Both proposed parameters allow to set the same (matching) sequence of ranking strains. Sensitivity of the method: identification of differences in BS between the generations of the bifidobacterium strain, the possibility of analyzing samples of concentrates with a volume of 2 µl.

The development of the analysis of BS and EPS as non-protein components immobilized in gel and on blot [24, 29, 30] included a sequential multistep procedure "IEF—PAG in pH gradient 4-8—Electroblotting—Staining with fluorescent dye—Reconstruction of visual patterns of GC location areas", which allows to obtain and enhance the discreteness of the separation of BS and EPS types and to establish/ recognize the details of the physico-chemical structure of the GC regions.

Results

The conditions for the detection of FL of BS and EPS in PAG plates and on blots were optimized. The differences in the total activity of BS were due to differences in the FL patterns of high-molecular-weight BS and EPS in PAG causing local swelling in the near-

neutral and cationic regions, respectively. BS were characterized by genus and species specificity, and were electrophoretically associated with a set of proteins with pI 7.2-7.6 or pI 7.4-8 and a molecular mass of 21-33 or 18-30 kD in the case of lactobacilli or bifidobacteria, respectively. The observed patterns allowed to judge the nativity or degradation of BS and EPS. The sensitivity of the method is up to 7 µl of the CF supernatant concentrate per track at IEF-PAG.

In general, the proposed algorithm for the systematic study of BS and EPS included: determination of the total activity of BS of CF concentrates; identification of types of BS and EPS according to the data of a pattern multistep (see above) analysis with effectively separated BS and EPS in the gel and on the blot. The combination of both proposed methods for determining the activity and physico-chemical properties of BS and EPS made it possible to evaluate the ingredients of the total BS activity of CF concentrates of probiotic bacteria. The results indicate the presence of at least 4 physico-chemical types of BS and EPS: minimally pronounced (residual - background); near-neutral BS of "lactobacillar" or "bifidobacterium" types; pronounced cationic EPS of bifidobacteria as additional contributors to the total activity of BS concentrates of CF supernatants.

Conclusion. Based on the results obtained, we formulated ideas about latent high-molecular multicomponent BS and EPS and their active low-molecular derivatives (recorded during IEF-PAG followed by blotting in the case of analysis of the initial supernatants of CF of GPB before concentration). It has been established that the total activity of BS of CF can be regulated: a) by production of low molecular weight near-neutral and cationic GC; b) by additional production of physico-chemical sets of high molecular weight BS and EPS by strain; c) by the presence of depolymerases, including endo-glycosyl hydrolases/ endopolysaccharidases. It is proposed to use visual patterns of BS and EPS in combination with associated proteins for genus, species and strain phenotyping. An experimentally supported hypothesis is proposed on the reversibility of the assembly and degradation of the system of GC—proteins, which involve interactions of the system Lectin—(GC type).

Prospects of the proposed methods for the study of polymeric GC: screening of soluble and immobilized BS and EPS, their expression and consumption under cultivation and storage conditions; screening of factors of increase/decrease in BS activity (selection of medium, its design, selection of cultivation conditions, advanced analysis of BS activities in high–molecular associate concentrates of biological fluids - special physical conditions similar to

plasma and serum); control of expression and production of depolymerases, predominant endohydrolases with different specificity; controlled production of activated low molecular weight BS and EPS (derivatives of different types of high molecular weight GC) for their further use as synergistic antimicrobial, prebiotic, anti-adhesive and other biologically active ingredients; further postgenomic standardization of GPB strains with respect to the severity of BS and EPS types.

2.3. Diagnostic and prognostic visualization of the systemic components of the C4 complement component of patients

Studies of GC–bi- and polyfunctional carbohydrate-containing natural substances and synthetic compounds are important for biomedicine and biotechnology, especially in connection with recognition processes at the molecular level [24, 26, 31-35]. At the same time, the novelty and significance of the results obtained are due not only to the high sensitivity of the devices and their ability to simultaneously monitor a number of parameters, but also to the development of new and improved traditional methods, methodologies and experimental approaches.

The HCS is a vivid example of the implementation of a variety of protective mechanisms for the recognition of GC within the framework of innate immunity [36-41]. The C4A and C4B isotypes of the C4 component of the HCS are able to activate under disease conditions, to release highly reactive C4Ab and C4Bb fragments as a result of limited proteolysis (the ability to interact with AB to C4 remains) with exposed reactively active bonds and covalently bind (exhibit functional activity, be functionally active, characterize the functional activity of C4A and C4B isotypes) to closely localized GC-targets. The targets are the protein part of the GC (in the case of C4Ab) or the carbohydrate part, including carbohydrate antigens of microorganisms (in the case of the C4Bb fragment). At the same time, a system of multiple forms (C4A/C4B-isotypic and subisotypic) - patterns of diagnostic significance is formed in the patient's blood, in which the relative severity of isotypes is determined by the hereditarily determined degree of deficiency of the C4A/C4B isotype (complete or partial), and the spectrum of related targets characterizes the features of the patient's disease.

The aim is to summarize our progress in the field of studying GC-binding systems using the example of the C4 component of the HCS.

Materials and methods. Serums of patients with autoimmune and systemic infectious diseases were used. Desialyzed serums (see below) were separated by IEF-PAG in an acidic pH gradient (3-5 or 2-6) without the presence of urea and sucrose, electroblotted onto a membrane sandwich: *Durapore* hydrophilic membrane (Millipore) and *Immobillon P* hydrophobic membrane (Millipore). The distribution of multiple immunoactive forms of C4 associated with targets on the blot was shown immunochemically using polyclonal rabbit AB to C4 labeled with horseradish peroxidase, which was shown in the presence of a chemiluminescent substrate. The kinetics of the CL of the obtained set of kinetic patterns on the blot was recorded in a dark chamber (*Dark Room*) of the *BioChemi System* (UVP, Calif., USA) using a *Bromide Ethidium* light filter.

Results and their discussion

Desialation of GP by the example of C4A and C4B isotypes. This initial step is key in the multistep study of the C4 system and for the development of methods and methodologies for the study of blood and sialylated GC of patients.

Tasks to be solved: increasing the reproducibility of the desialylation method, reducing the electrophoretic microheterogenicity of GP and their complexes, increasing the reliability of the final results, visual control of desialylation to optimize the physico-chemical reaction factors for the selected sialidase preparation.

The principle of the GC desialylation method. The structures of the carbohydrate part of the human C4A and C4B isotypes are known - they are typical for the "serum" type GP and include predominantly pronounced sialylated Asn-glycans of the complex type, opposed to Ser/Thr-glycans of the "mucin" type. *Clostridium perfringens* bacillar sialidase (Sigma, USA) is capable to desialylate blood GP. The desialylated isotypes C4A (less acidic) and C4B (more acidic) separated by IEF-PAG are located within the pH 5-6 region in the form of relatively weakly expressed minimal sets of multiple forms. Under the conditions of disease, multiple complexes of functionally active C4A and C4B isotypes and characteristic (for disease) target GC are formed. Such resulting complexes are more clearly detected (compared with pH 5-6) in a more acidic region (pI 4-5). The use of "model" sera of patients (with known deficits of one of the isotypes established using the methods of micropanel enzyme immunoassay of functionally active C4A and C4B described above) to optimize the desialylation reaction led to the expected (by the type of known serum deficiency) visual CL

patterns on a blot with a well-reproducible arrangement for different sera of patients isotypes C4A (pI 4.0-4.3) and C4B (4.58-4.65) on the blot are pH7.

Description of the method. Blood serum samples (hemolysed sera containing intracellular catalase, which destroys hydrogen peroxide, a peroxidase activator, were not taken) were desialyzed at pH 7 initially at 56^{o}C for 30 minutes (coinciding with the time of thermodenaturation inactivation of CHS; as a result the availability of glycans for sialidase is increased against the background of increasing resistance of complexes to proteolysis; more over the antigenic properties of complexes with exposed C4 are improved). Incubation was continued overnight at room temperature in the dark (there is reason to believe that additional incubation may not be used during thermal incubation for 40 minutes). The stabilizer of sialidase was albumin present in serum (approximately 40 mg/ml; 10 mg/ml is sufficient). 2-4 µl of thermally stable (at 51-55 °C) neuraminidase of *Clostridium perfringens* (5 mg of protein in a 50 mM K-phosphate buffer pH 7 with 5 mM Na_2-EDTA) with 5 µl of *"Complete"* (proteinase inhibitors of all 4 large groups: Enzyme Classification 3.4.21-24) were added to 7-8 µl of serum (1 commercial tablet of inhibitors was dissolved in 1 ml of sterile saline solution).

Serum desialation was monitored by IEF-PAG, followed by electroblotting to the membrane. The isotypes were manifested by the conjugate of AT with peroxidase. Doubling the dose of sialidase led to the appearance of additional intermediate products between the positions of C4A and C4B on the blot, complicating the analysis of subisotypes. Inactivation of serum by HCL (10-fold dilution with distilled water) with a simultaneous 10-fold decrease in the dose of sialidase led to a significant decrease in the level of C4B (including in C4B-rich serum) and was not used in the desialylation procedure.

The *sensitivity of the method* is 0.3 µl of serum in the track on the PAG plate when detecting C4A (7 µl of serum to identify both isotypes), accurate determination of the complete deficiency of the isotype and subisotype.

Prospects of the GC desialylation method: breadth of application (natural and recombinant GP; eukaryotic extracellular, from animal biological fluids, GC of microorganisms); the possibility of using partially purified sialidases and combinations of sialidases with different specificities; investigation of the specificity of the selected/ studied sialidase using standard blood GP and other standard GC with known chemical structures; as a preliminary stage of

analysis of the carbohydrate composition and structure of GP glycans and other GC; reduction of microheterogenicity of GC pools and individual GP and other GC; reproducible controlled production of desialylated sera of patients with abnormal carbohydrate GP and other GC (potential biomarkers, including organotropic ones) for further study; standardization of the serum desialylation reaction for use in the process of desialylation of cells (erythrocytes [EC], leukocytes and others; desialylation efficiency is established by achieving maximum cytoagglutination with lectin, for example, ConA); increasing the hydrophobicity of uncharged GP and other GC and increasing their ability to react with AB.

Analysis of the C4A and C4B subisotypes. pH4.5(acetate buffer)-blot treatment-pH7 [blot-pH7] led to background alignment on the blot, regardless of the pH gradient initially used in PAG (the best result is due to consideration of the acetate-binding subsite of the catalytic site/horseradish peroxidase center). Prior to the pH 4 treatment of the blot, the diffusivity and low discreteness of the separating isotypes and subisotypes were strongly pronounced. After pH4 treatment, a significant increase in the discreteness of the subisotypes was achieved. Initially, the overestimated values of C4A/C4B in the case of blot-pH7 (due to an overestimated background in the C4A region) in the case of blot-pH4, the values of C4A(sum of subisotypes) / C4B(sum of subisotypes) better corresponded to the values of C4A/C4B determined by micropanel analysis of the functional activity of isotypes. Computer reconstruction of pattern details improved the detection of minor subisotypes. In controversial cases, additional control using intermembrane (from immobilon to immobilon) acidic electroblotting transfer of peroxidase activity made it possible to confirm the established location of the subisotypes. Pattern analysis made it possible to reasonably identify rare sera with a deficiency of both isotypes (and, accordingly, subisotypic sets of isotypes).

The results of subisotyping. Data were obtained on the visual patterns of the subisotypes of the C4A isotype (A1, A2, A3, A4 and A5 [A5 is revealed in the earlier kinetic picture of CL as a diagnostic form closer to the anode in cases of patients with systemic lupus erythematosus and antiphospholipid syndrome]) and the C4B isotype (B0, B1, B2 and B3) of sera of patients. It is possible to predict the risk of infectious diseases with deficits of subisotypes of the C4B isotype.

In the area of the location of the C4A and C4B isotypes in the direction from C4A to C4B, the gradient of the moiety of expressed carbohydrates in GC (on the example of GP type of

GC) was revealed by the degree of increase in the severity of the carbohydrate part in GC. The gradient could be visually controlled at the subisotype level.

The results indicate the presence of several kinetically recorded diagnostic patterns (identified at different, albeit close, time intervals for the detection of CL), which expand the possibilities for more accurate diagnosis of the disease and assessment of the patient's condition. The results indicate the possibility of monitoring serum subisotyping in the process of rehabilitation or deterioration of the patient's health.

In general, isotyping and subisotyping of C4 on the blot before and after pH 4 treatment complemented each other (taking into account the contribution of subisotypes with increased hydrophilicity on the blot-pH7 and with increased hydrophobicity on the blot-pH4) and made diagnostic and prognostic conclusions more reliable.

Conclusion. The results indicate the possibility of assessing the state of the body's innate defense (detecting deficiencies of the C4A and C4B isotypes of CHS in a new, more sensitive diagnostic area with high discreteness of bands), simple visual pattern control and monitoring of adaptive processes of expenditure/consumption of functionally active C4A and C4B isotypes and their subisotypes interacting with GC, depending on the types of human systemic autoimmune diseases. Based on the results obtained, a concept and algorithm for a complete diagnostic and prognostic pattern of the system components of functionally active C4 sera of patients is proposed, involving a sequential analysis of the isotypes and subisotypes of the C4 component of the CHS.

Prospects of the algorithm for determining visual patterns of the patient's C4-dependent system: possibilities for further study of the subisotypic composition of C4A and C4B isotypes in patient sera (identification of new diagnostic forms and their combinations, kinetic pattern combinations, detailed study of the biochemical composition of subisotypes); possibilities for further strengthening the discreteness of GP forms in IEF-PAG and/or blot conditions (use of monoclonal AB, immune sandwich); the possibility of additional purification when obtaining C4A and C4B isotypes (and their subisotypes) immobilized on the membrane; the possibilities of immobilization and purification of subpolyclonal AB corresponding to the main subisotypes; the possibilities of practical use of the obtained immobilized biocatalysts and their cascades, including on the basis of functionally active (signaling) fragments of C4A and C4B isotypes.

2.4. Determination and analysis of the antagonism of microbes and their metabolites in BF

The issues of surface-cellular and intercellular recognition, the involvement of lectins, adhesives, enzymes and other recognition signals in them are relevant.[42,43] At the same time, the choice of cellular and intercellular systems and factors with recognition functions is important for laboratory modeling. We have conducted and developed studies using erythrocyte and yeast systems[44-49] and other yeast-like and lactobacillar systems of human biotopes involved in biofilm formation (BFF).[26,50-65] The aim of the work is to summarize our progress in the study of BF in various model conditions.*Materials and methods.* Systems based on erythrocytes (EC) and yeast cells were used. Trypsin (Spofa, Czech Republic) 10-15 mg/ml in HCl pH 3, was added to 40-50% (by volume) to EC suspension (without admixture of leukocytes) in PBS pH 7.4 (trypsin as 1 mg/ml final concentration) for 2 hours at 37^{o}C or up to a concentration of trypsin as 0.1 mg/ml (EC incubation for nights at 4^{o}C). Both variants of trypsinized EC (trypEC) gave a similar result of lectin hemagglutination (HA). EC was asialylated with sialidase (Enzyme Classification 3.2.1.18) of *C. perfringens* (Sigma, USA; cleaves off mainly the residues of NeuNAc-α-2,8-; desialylation is described above). Desialylated EC (asiaEC) of AII(+) blood groups were obtained (with predominantly exposed residues of N-acetyl-D-galactosaminylpyranoside [D-GalNAcp] imitating the surface of mucins). trypEC and asiaEC were washed with PBS pH 7, stored at 4^{o}C, and used for a week.

Cytoagglutination (CA) was studied in a round-bottomed 96-well micropanel (preferably polystyrene with increased hydrophobicity of the bottom of the wells) before and after sensitization of the wells with lectins. 0.5-1% EC suspension or stable suspension of commercial yeast in PBS was added to the lectin preparations sorbed in wells in serial dilutions. The breeding of Con A (Sigma, grade IV) served as a control. BF and CA titers in the volume of 50-100 µl/well were visible after 30-60 minutes (25^{o}C), a stable pattern after a day (4^{o}C). The preservation of the HA pattern took several weeks (after 3-4 weeks, the pattern practically did not change) at 4^{o}C, including during regular resuspension of BF and removal of appendages. The stability of erythrocyte BF (the absence of hemolysis and the appearance of a green patch (due to verdoglobin) with a pH shift from 7.4 to 8.3), as well as the shelf life of the systems decreased in the series: trypEC > asiaEC > native EC (the presence of their own surface active proteases).

Coagglutination with erythrocyte pairs was used for the manifestation of lectins in subagglutinating concentrations, an increase in the CA titer against the background of a decrease in the influence of suprasorbent factors (agglutinate-absorbing activities, nonspecific panagglutinins with low CA titers). After establishing the initial HA pattern in a series of dilutions of lectins, the attachments were removed from the wells and trypEC or asiaEC were added to the lectin-sensitized bottom (bound Ec and free polystyrene) to form layered assembly homo- or heteroparticles (trypEC + trypEC, asiaEC + asiaEC, trypEC + asiaEC or asiaEC + trypEC). An additional way to increase the sensitivity and direction of coagglutination is the addition of lectin-sensitized trypEC or asiaEC. BF based on yeast-like micromycetes in cultures. Identified strains of *Candida* and *Lactobacillus* isolated from the intestinal and urogenital tracts of humans were used, the BF formation of which was studied on agar with *Saburo* medium (in the presence of discs with lectins or antimycotics; after 2 days under standard conditions and under conditions of cold prolonged stress) or in flat-bottomed polystyrene micropanels in MRS medium (D-Man–Rogosa–Sharpe agar) (without or in the presence of lactobacilli of the same biotope; after 2 days at 37^{o}C). The initial suspensions of microbes had a turbidity of 1 unit on the *McFarland* scale. Optimized ratios of microbial suspensions and media were used in the wells of the micropanel. BP was treated with gentian violet, which was extracted with a solution of acetic acid. The extracts were transferred to a micropanel and the absorption was measured on a reader through a 620 nm light filter.

Proprietary preparations of lectins from cultures of probiotic strains of lactobacilli and bifidobacteria, phytolectins from higher and lower plants of medical importance and soluble linear pseudopolysaccharide GC based on PA (as carrier) were used as modulators of CA and BF. HA (0.5-5.0 mcg/ml, in PBS) contained exposed clusters of short (from one or two carbohydrate residues) carbohydrate antennas, departing from the PA chain and mimicking glycans of the mucin type (www.lectinity.com).

BF was recorded under optimized conditions in live image mode in *BioChemi System* (UVP, Calif., USA), photographed and scanned. The resulting digital images, photographs and scans of BP and color solutions in micropanel were edited using a computer. Cellular systems did not give satisfactory results when using lectin-sensitized blots.

Results And Their Discussion

BF based on EC and yeasts in CA reactions

Imitation of cells and cellular properties by lectins was observed in cases of cellular suspension lectin forms (CSLF), subpopulations of cells as sets of CSLF, coagglutination involving subpopulations of cells as sets of CSLF, directed solid-phase layered assembly of subpopulations of cells as sets of CSLF. Increased sensitivity of CA was achieved by repeated titration (re-titration) after removal of well attachments; two-dimensional titration using a combination of cellular systems (including coagglutination) and lectin-sensitized wells; analysis of the clot dissolved activities (CDA) of preparations (obtained two-dimensional mosaics of circles of incomplete [up to the "point" pattern in well] HA in micropanel corresponding to dilutions significantly exceeding the classical HA titers); layered assembly (including in lectin-sensitized wells); visualization of CA in wells with dilute EC suspensions (less than 0.5% by volume in final concentrations) by converting HA well circles into ellipses or adding EC hemolysate to yeast without affecting the lectin titer (using the example of ConA - [predominantly mannan]–binding phytolectin, intensively interacting with yeasts), computer editing of digital patterns.

Cellular systems for simultaneous analysis of lectins and their inhibitors in CA included systems with initial incomplete CA in wells, as in the case of trypEC (EC with reproducible incomplete well self-agglutination in the form of circles of incomplete [not 100%] HA for the study of the contribution of surface cell lectin glycophorins and exogenous lectins; addition of methyl-α-D-galactopyranoside [Me-α-D-Galp] to a suspension of the EC (BIII blood group with exposed D-galactose residues) turns incomplete circles into a dot; a system for testing any biologically active GC).

Increasing the diversity of CA with drugs in the experiment

In solution during the analysis of CDA, panagglutination with colored impurities, incomplete well HA with hydrophobic proteins such as bovine serum albumin, the contribution of polysaccharides to HA according to the principle of "All (100% HA in wells)—Nothing (control in the form of a point BF of EC)" [using the example of a crystalline carbohydrate-containing lectin from wheat germs - WGA], the transformation of hemoglobin (red in hemolysis-protected BF) into modified verdoglobin (green in hemolysis-protected BF) (verdoglobin with only two chemical bonds broken, but preserved in the iron atom and globin molecule) under the influence of NAD-dependent oxygenase (varying intensity of the green

color of the solution in wells with variously expressed residual close to minimal BF), peroxidase-like of hemoglobin activity, especially in conditions of alkaline pH during the transition from pH 7.4 to pH 8.3 against the background of the presence of endo- and exogenous peroxides – initiators of peroxidase activity, oxidoreductase crosslinking BF activities, involvement of proteinases of the surface of native EC in hemolysis at pH 7.1.

In the solid phase - with increased selectivity of CA, complete or partial removal of BF, analysis of BF protection, the appearance of cementation (multipoint non-covalent and with the formation of chemical crosslinking) of BF, escalation of aggregation in resuspended BF (increasing the degree of irreversibility of BF resuspension during storage), detection of sets of types of aggregates in resuspended BF with regularly removed attachments (the appearance and elongation of rod-shaped prominences along the edges of the circles of the lunate HA, and other aggregation inhomogeneities.

The results indicate the presence of molecular microreflex matrices (realized in the observed macro-events) - branched and extended [linear] supramolecular assemblies as initiators of CA escalation). The assemblies are sensitive to GC: they were inhibited mainly by GalNAc-PA (mucin mucin simulator), Man-PA (yeast mannan and mucin mucin mannan simulators), and others, depending on the characteristics of the assemblies. GC had the ability to "dissolve" BF (hemagglutinates) after addition to BF and their resuspension.

To model BF in directed affine layered assembly (obtaining gradients: heterocellular, including in combination with lectins and cytokines, cellular cytokine producers), in the irreversibility of assembly in time, protection or degradation of BF by cell-sorbed effectors. The conditions for the inhibition by lectins of probiotic bacteria of fungal assemblies initiated by lectins of BF have been established (using the example of coagglutination variants of HA). The results indicate antagonistic efficacy against fungal lectins, primarily Man-PA/Man-6-phosphate-PA-specific constituents of human probiotic bacteria lectin systems.

BF in cultures of yeast-like *Ascomycromycetes* (on the example of *Candida*) and GPB (on the example of lactobacilli of the same biotope).

Scanning and editing of micropanel-stained BF (as the sum of BF of sorbed cells at the bottom and BF of the side walls in each well) allow quickly evaluate the BF of microbes and their combinations and select the optimal ratios of *Lactobacillus* and *Candida* in the medium.

Cultivation of mixed and monocultures in optimized variants allows further selection of conditions for the identification and rules for the establishment of leader strains in interacting pools of lactobacilli and *Candida*.

In the "Growth+BFF" reactions, the phenotypic relationships between *Candida* and lactobacilli between species, subspecies and strains were identified and calculated, including taking into account the antimycotics/antibiotics sensitivity of interacting microbial pools. An assessment of the effect of *Candida* pools (or lactobacilli) on the nature of the ranking of the severity of BFF by mono- and mixed cultures of lactobacilli (or *Candida*) gave additional independent evidence in favor of the functional/metabolic proximity of *C. albicans* and *C. tropicalis*, the effect of combinations of *Lactobacillus* species on BFF by intraspecific subpopulations of *Candida* strains, the existence of phenotypically different (similar in the reaction of BFF) two subpopulations of *C. albicans* and two subpopulations of *C. tropicalis* in a population urban biotope dependent on biotope lactobacilli (similar in the reaction of BFF) two subpopulations of *C. albicans* and two subpopulations of *C. tropicalis* having diagnostic significance. It is possible to assess the leadership (selection from several strains of a population of GPB) of a probiotic candidate strain of lactobacilli showing directed antagonism against the *Candida* species/strain.

According to our data, lectins of probiotic bacteria mimic many (main) beneficial key reactions of probiotic cells. The surface of lactobacilli is characterized by sets of GC-recognizing and binding lectins, and lactobacilli themselves are CSLF affine oriented by navigator lectins to other macro- and molecular targets and in the processes of directed assemblies in the composition of BF. With prolonged contact of lectin disks of probiotic bacteria with *Candida* arrays and their BF on agar, a multi-stage predictable degradation of *Candida* is observed, including a cascade destructive effect of bifidobacterial lectins (mainly Man-PA/Man-6-phosphate-PA-preferentially specific; action mainly in borderline areas unfavorable for survival of the communicative body of yeast-like fungi, *Ascomicromycetes*) and then lactobacillar lectins (mainly GalNAc-PA-specific [mucin-binding]; action mainly in the central regions of the communicative body of ascomicromycetes favorable for survival), as well as strain-dependent wave cascade macrolysis of a continuous array initiated from a narrowly localized zone in predictable directions. The zones of destruction and lysis of the *Candida* massif initiated by lectins showed varying (depending on the characteristics of the territory of the interaction area) resistance to colonization by molds - *Aspergillus*, *Penicillium*

and *Mucor* (against the background of preservation in zones of complete bactericidal activity). Interactions in the system "(Lectins of probiotic bacteria)/lactobacilli – *Candida*" occurred in accordance with the concept of a communicative self-controlled "body" of the ascomicromycete, as well as the concept of a multi-node network-linked microbiocenose biotope. This made it possible to carry out a diagnostic and prognostic assessment of events in the system, to design functional models of practical importance for laboratory and medical biotechnology.

Conclusion. Laboratory biotechnologies based on simple cellular systems are proposed, using the principles of hemagglutination and CA methods, analysis of microbial arrays and BFF, taking into account protein recognition of GC.

The catalog of digital photographs confirms the cases of CA and BF listed and described above**.**

3. GENERAL CONCLUSION

Laboratory biotechnologies based on cellular systems, cell-free biologics and their combinations are proposed for widespread use. The methods and experimental techniques, methodologies and approaches are indicated, including those that open the way to the study of new natural substances, increase the sensitivity of research, and provide high information content of visual digital patterns. Micropanel and blotting methods (qualitative, semi-quantitative and quantitative; erythrocyte and yeast) are close to biochip in sensitivity. The microanalysis is supported by recording the results of the interaction between proteins and GC by fluorescent and chemiluminescent methods in live image modes. The possibilities of evaluating functional visual patterns associated with BF processing and their regulation by (oligo)peptides, low-molecular, high-molecular GC-containing factors and signals (including Quorum Sensing, (QS) and Cross-Talking) are promising and perspective. Methods-based methodologies and algorithms proposed for the study of combined cellular systems and BF have prospects in the current routine laboratory analysis of antagonistic cellular and competitive metabolic components of human body biotopes. The aforementioned results, conclusions and based on them concepts indicate broad and deep prospects for studying recognition involving lectins and GC at all levels of human organization, for the development of innovations in medical biotechnology. Conclusions and concepts allow to consider and apply results in a broader field of investigation.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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