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POTENTIAL OF THE MINIMAL SYNBIOTIC SYSTEM INVOLVING PROBIOTIC LECTINS, METAL CATIONS, AND SYNTHETIC POLYMERIC GLYCOCONJUGATES

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ABSTRACT

Insulin 1 ml syringes (20 microliters-scale) were used for cultivation of probiotic microorganisms in the symbiotic conditions. The synbiotic system in syringes included lectins from the human intestinal probiotic bifidobacteria, Li^+ cations and anionic natural or synthetic polymer glycoconjugate (mimic natural glycosaminoglycans). The visual growth of colonies of bifidobacteria sorbed on the inner wall of syringes in the presence of synthetic glycosaminoglycans was demonstrated in direct proportion to the dose of the added preparation of endogenous bifidobacterial lectins. Separation of the preparative amounts of produced gel-like exopolymer substances (the bottom), adsorbed colonies (the middle space) and soluble waste products of bifidobacteria has been achieved from syringes. The results point to the prospects of using insulin syringes as probiotic minibioreactor. Probiotic lectins can be served and used as endogenic (for the same probiotic strain tested) or exogenic (for a number of probiotic strains) prebiotics.

KEYWORDS: Insulin syringes; *Bifidobacterium*; synbiotic; bioreactor; adhesion; biofilm, microecology.

ABBREVIATIONS

B	biotin
BLS	bifidobacterial LS

BM	*Bifidum Medium*
EPS	exopolymeric substances
GC	glycoconjugate(s)
IEF	isoelectrofocusing
IS	insulin syringe(s)
LPB	lectins of probiotic bacteria
LS	lectin system(s)
ml	milliliter(s)
PA	polyacrylamide
PAG	PA gel
pI	isoelectric point(s)
PL	probiotic lectins
µl	microliters

1. INTRODUCTION

Probiotics represent a strategically important object for biotechnology and medicine.[1-15] New perspective regulators and modulators of probiotics include probiotic lectins (PL), which mimic the main functions and actions of probiotics, represent a new class of pathogen disruptors and play an important role in the vital activities of human probiotic bacteria.

On the one hand, modeling the behavior of probiotic bacteria in small volumes is convenient in the laboratory, allows you to quickly obtain results and refine technologies with minimal reagent costs and losses, facilitates visual assessment using quantitative scale, makes it possible to compactly preserve the best experiments for a long time, and reduces the consumption of materials and reagents. Thus, insulin syringes (IS) are standard, micro-quantitative (µ-scale), available, sterile, compact, hermetic, transparent, chemically inert, physically stable, inexpensive, biocompatible (designed for humans), convenient as flow-through mini-bioreactors, storage tanks, and simulators of the constituents of the intestinal environment. Plastic syringes with a built-in needle make it possible to eliminate the "dead space" in which a certain amount of solution remains in a conventional syringe with a removable needle after injection. Such syringes can be used repeatedly. The inner surface of commercial IS is coated with heparin in combination with Li^+ cations, which makes IS a convenient model for studying synbiotic processes dependent on metal cations and

glycoconjugates (GC). In addition, small quantities of microbial cells and reagents can be used in conditions of IS.

On the other hand, lectins of probiotic bacteria (LPB) belong to a new class of multifunctional systemic metabolites of cultures of human probiotic bacteria, their natural consortia, and various variants of mono- and multi-strain probiotics. LPB contain metal cations, are capable of binding Li^+ cations and desorbing from the surface in the presence of Li^+ cations. LPB are characterized by co-functioning with (bio)polymer effectors – natural and synthetic GC. LPB with isoelectric points (pI) at pH 5-6 and pI > 7 are functionally linked to enzymes of different classes or exopolymer substances (EPS), respectively.

Human probiotic bifidobacterial lectin systems (BLS) reveal GC-recognizing and binding properties providing multifunctional realization of useful for organism reactions supporting biotope microbiocenosis' healthy balance. BLS co-function to innate and adaptive human recognition systems involving own lectin systems (LS) of protective significance. BLS possess the selective adhesiveness, can be bound to metal cations (also Li^+) and polymeric GC, reveal properties of carriers and delivery agents of GC type prebiotics, antioxidants, antimicrobials, therapeutics and other protective agents.

The aim of the work is to study the vital activity and behavior of probiotic strains of bifidobacteria in IS in the presence of exogenous non-bacterial or endogenous bacterial (produced by bacteria of the same species or strain) modulators; to propose a model of the minimal synbiotic system in IS, including BLS and Li^+ cations, for screening multi-fuctionality of each type GC used (their modulator and prebiotic effects), and to summarize a potential of such synbiotic system.[16-18]

2. MATERIALS AND METHODS

The work used industrial probiotic strains of bifidobacteria (*Bifidobacterium adolescentis* MS-42, *B. bifidum* No 1, and *B. gallinarum* GB) from the Collection of Microorganism of the *G.N. Gabrichevsky* Research Institute of Epidemniology and Microbiology. Bifidobacteria were grown on the (*Bifidum Medium* [BM], Obolensk, Moscow Region). Bacteria were grown for 18-24 h at 37°C on a rocking chair in sterile glass jars with a volume of 0.5 liters, as well as in the vertically placed IS with a volume of mixture 1 ml. One ml of mixture (in completed IS) contained 910-955 microliters (µl) of degassed sterile medium + probiotic as

bacterial starter culture or sourdough (*G.N. Gabrichevsky* Institute) + modulation factor 5-50 µl.

The tested modulation factors included LiCl, exopolymer substance [EPS- a viscous high-molecular fraction of a 100 times concentrated supernatant] or BLS [a fraction containing acidic or alkaline types of the BLS, including lectins from the same bifidobacterial strain tested in IS]). The working dilutions of the factor tested were 1 : 20 (external factor 50 µl + liquid phase 950 µl in IS) and 1 : 200 (external factor 5 µl + liquid phase 995 µl in IS). Controls were without regulator factor.

Hermetically sealed insulated mixtures (bifidobacteria in liquid phase) were incubated at 37^{o}C up to 18 days in BM in anaerobe sterile conditions in IS whose inner surface was coated with a complex of heparin-Lithium cations. BLS were isolated from acidic and alkaline fractions 27-220 kD (pI 4-8) using isoelectric focusing (IEF) in gradients of pH within interval pH 3-8 in the plate of polyacrylamide (PA) gel (PAG) in the presence of 7-8 M urea and 5% saccharose. Electroblotted proteins and EPS were evaluated using *SYPRO Ruby Protein Blot Stain* (Bio-Rad Lab). BLS were characterized by binding to GC-PA-biotin (Sugar-PA-b, www.lectinity.com) manifested with streptavidin-peroxidase and chemiluminescent substrate for peroxidase. Blot fluorescence and chemiluminescence as well as pictures in IS were registered in the *Dark Room* of *BioChemi System* (UVP, USA) in the live bio-imagine regime (a real time). Residual gel biomass produced in IS was evaluated after elimination of liquid phase.

A synbiotic system in IS included BLS from the intestinal probiotic bifidobacterial strains, Li^{+} cations and acidic anionic natural or synthetic polymer GC (www.lectinity.com) mimic natural glycosaminoglycans.

The following processes were monitored: a) the initiation zones of individual colonies, the growth of their mass, the distribution of adsorbed colonies and biomass; areas of turbidity; stages of vital activity. The distribution of colonies and biomass within the IS was recorded in transmitted light by focusing on the interstitial inner space of IS (Figure 1-Left), and the distribution of adsorbed, including flocculated polymeric biomass, was photographed after removing non-wall-sorption material (its volume can be measured) from the IS (Figure 1-Right) in the *Dark Room* camera of the system *BioChemi System* (UVP, USA). Computer editing of the digital photos was carried out.

3. RESULTS

3.1. Factors influencing the bifidobacteria in IS

3.1.1. The effect of BLS on bifidobacteria in IS

BLS were characterized by different in pI-distribution compared to lactobacillar LS. BLS reveal affinity to acidic GC (similar to heparin) such as $(Gal\text{-}3\text{-}sulfate)_n$-PA or $(mannose\text{-}6\text{-}phosphate)_n$-PA. Acidic BLS (within pI 4-4.5; possessing higher adhesive, aggregating and haemagglutinating properties than alkaline BLS) revealed higher intensities and prolonged affinities to these GC compared to alkaline BLS. Acidic BLS were characterized by a pronounced affinity for anionic GC (with exposed residues of sulfated galactosides [especially] or mannose-6-phosphate), mimics of naturally glycosaminoglycans.

3.1.2. The effect of Li^+ cations on bifidobacteria in IS

1) Li^+ cations (originally covered on heparin surface within IS) randomly and lately initiated forming 1-2 colonies.
2) Additional Li^+ cations (15-150 mM) initiated early appearance of colonies, increased multiplicity of colonies (up to 10 in a syringe, without their intensive growth) in a Li^+-dose dependent manner.

Thus, Li^+ cations increased the number of bifidobacterial colonies on the heparinized surface in a dose depended manner.

LiCl (15 mM) initiated the maximum number of adhered colonies throughout IS. 150 mM LiCl stimulated the appearance of massive pronounced colonies without differentiation at the top of IS.

3.1.3. The effect of EPS on bifidobacteria in IS

Bifidobacterial EPS endogenically stimulated an early (within the first three days) increase in the mass of bifidobacteria, mainly in the lower half of IS, as well as a mass with pronounced adhesion in the middle of the syringe.

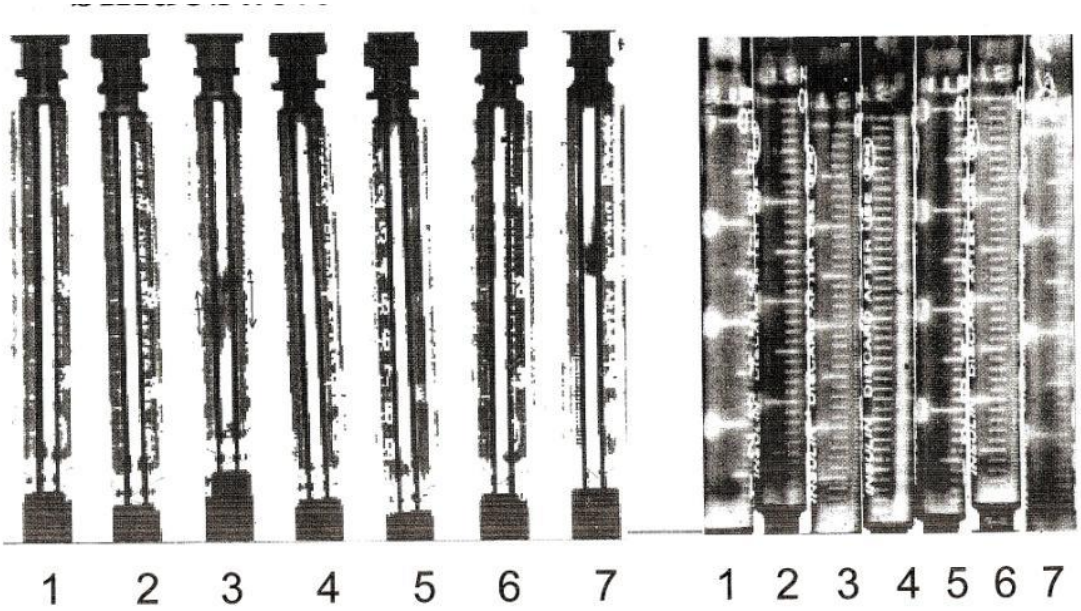

Figure 1: Effects of the addition of external physico-chemical factors on bifidobacteria in conditions of IS.

Left: Samples of *Bifidobactecrium* GB (race 4) visible colonial mass (No 3 > No 7 >> No 4, No 6) distribution on internal walls of the middle space of IS. Involvement of acidic endogenic BLS is expected as the dominant factor compared to the action of alkaline endogenic BLS.

Right (the same numbering and order of IS as on the left**)**: Examples of bifidobacterial gradient (bottom—top) gel biomass distribution along syringe internal walls in direction from bottom to tip (No 2 = No 5 > No 7 > No 1). Involvement of alkaline BLS (associated to EPS) is argued by IEF-PAG in the gradient of pH and electrophoresis-PAG in the *Laemmli* system.

3.2. The main features of the bifidobacterial (auto)synbiotic process in IS

* BLS induced increasing both adhesision of colonies and gel biomass (non-protein exopolymers associated to proteins) of bifidobacteria (on example of *Bifidobactecrium* GB, Figure 1).

*Originally adsorbed on heparinic IS internal surface, Li^+ cations practically do not initiate the forming colonies.

*Additives of external Li^+ cations (15-150 mM) initiate early appearance of colonies, increased multiplicity of colonies in a dose dependent manner.

* Acidic and alkaline BLS participated in each adhesive colony biomass growth in the middle of IS or accumulation of the bottom localized EPS gel biomass, respectively.

* During prolonged incubation of serial cultures adhesive complexes (BLS-Li^+)-(acidic GC imitating natural glycosaminoglycans) kept ability to realize main functions of exposed ingredient contributors within IS.
* As a result of aforementioned data, adhesive directed BLS-Li^+-GC complexes (adhesive assembled LS) are involved in both support of bifidobacterial proliferation and stimulation of biomass growth on the sensitized anti-coagulant biocompatible anaerobe surface of the mini-bioreactor.

4. CONCLUSION

Results indicate that synbiotic actions in IS model of bioreactor involve solid phase functionally coupled complex of acidic BLS, metal cations and acidic GC on the IS internal walls. The potential of the proposed synbiotic minibioreactor (biocompartible to human biological fluids) include:

*screening prebiotic and therapeutic GC in the presence of BLS and Li^+ cations;

*screening endogenic microbial lectins as prebiotics;

*study of the peculiarities of microbial metabolism, depending on the medium and surface (including for testing new nutrient media and their factor-enriched additives);

*investigation of a wide range of biological properties of synthetic and natural GC (to identify and optimize the best conditions and for further GC standardization), including testing GC combinations with other type prebiotics;

*detection of the traces of biologically active O_2 in bacterial culture along cylindrical extended narrow small volumes (using bifidobacterial communicative body as a sensor of O_2);

*IS as contributors to design of probiotic bacteria communicative body;

*results (patterns) can be stored in IS, numbered along the axes of the rectangle in the form of "honeycombs";

*results indicate prospects of mini-bioreactors in simulation of important for life sensor properties of synbiotics;

*search of effective combinations of both bifidobacterial strains and regulation factors;

The described synbiotic bifidobacteria-stimulating system in IS, in which an important role is assigned to the co-functioning of glycosaminoglycan(s)-coated plastics, PBL as LS, and Li^+ cations, justifies the prospects for using metal-GC coatings of organic polymers and materials;

Disclosure of conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Bovkun GF, Ivanov II, Pospelova VV, Aleshkin VA, Afanasiev SS, Lakhtin VM, Levchenko TA. Biological characterization and prospects of application of a new bifidobacterial strain as an ingredient of probiotics. *Vestnik Rossiyskoy Akaddemii Meditsinskih Nauk (Moskva),* 2006; 3: 26-31. (in Russian)
   https://pubmed.ncbi.nlm.nih.gov/16734340/;
   https://www.elibrary.ru/item.asp?id=9182581
2. Patent RU 2 301 828 C1 (Published 2007.06.27). Bovkun GF, Vashchekin EP, Pospelova VV, Amirkhanova AM, Lakhtin VM. Strain *Bifidobacterium gallinarum* GB, used to produce bacterial preparations, biologically active feed additives, fermented and unfermented feeds intended for the prevention and treatment of intestinal diseases of young poultry. (in Russian)
   https://yandex.ru/patents/doc/RU2301828C1_20070627
3. Lakhtin M, Alyoshkin V, Lakhtin V, Afanasyev S, Pozhalostina L, Pospelova V. Probiotic lactobacillus and bifidobacterial lectins against *Candida albicans* and *Staphylococcus aureus* clinical strains: new class of pathogen biofilm destructors. *Probiotics and Antimicrobial Proteins*. 2010; 2(3): 186-96, DOI: 10.1007/s12602-010-9046-3. WOS:000209417300006.
   https://elibrary.ru/item.asp?id=17816861;
   http://link.springer.com/article/10.1007/s12602-010-9046-3 ;
   http://link.springer.com/article/10.1007%2Fs12602-010-9046-3
   http://link.springer.com/article/10.1007%2Fs12602-010-9046-3#page-1;
   https://mapofscience.ru/scientist/59925606/articles?clear=all&&amount=10&type=PUB&page=1&scientists%5B%5D=59925606

4. Lakhtin M, Lakhtin V, Bajrakova A, Aleshkin A, Afanasiev S, Aleshkin V. Lectin systems imitating probiotics: potential for biotechnology and medical microbiology. *In: "Probiotics 2012", Edited by EC Rigobelo.* New York, InTech, 2012; 417–32. http://dx.doi.org/10/5772/3444
5. Lakhtin MV, Lakhtin VM, Afanasiev SS, Bairakova AL, Karaulov AV, Afanasiev MS, Aleshkin VA. Mobile synbiotope microbiocenosis against pathogens. *Bulletin of Eastern-Siberian Scientific Center of SB RAMS (Irkutsk, Russia),* 2016; 3(Part 2): 168-73. (in Russian) http://byulleten.com/arkhiv/all/
6. Lakhtin MV, Lakhtin VM, Afanasiev SS, Aleshkin VA. Mucosal Opened Cavities as the Organ of Increased Resistance and Effectiveness. *Journal of Advances in Biology & Biotechnology (JABB),* 2016; 10(3): 1-10. DOI: http://dx.doi.org/10.9734/jabb.
7. Lakhtin VM, Lakhtin MV, Davydkin VY, Melikhova AV, Davydkin IY, Zhilenkova OG. Antimycotic systems of bifidobacteria. *World Journal of Advanced Research and Reviews (WJAAR),* 2021; 9(1): 69-74. e-ISSN 2581-9615 https://wjarr.com/content/antimycotic-systems-bifidobacteria; https://wjarr.com/sites/default/files/WJARR-2020-0496.pdf
8. Lakhtin VM, Lakhtin MV, Davydkin VY, Melikhova AV, Davydkin IY, Zhilenkova OG, Klimova EV. Antibiotic like systems of bifidobacteria and lactobacilli. *American Scientific Journal (ASJ),* 2021; 44-1: 4-8. Published: 2021-01-13. DOI: 10.31618/asj.2707-9864.2020.1.44. ISSN 2707-9864. https://www.elibrary.ru/item.asp?id=45693120; https://archive.american-issue.info/index.php/asj/article/view/3/2; https://archive.american-issue.info/index.php/asj/article/view/33/26; https://american-issue.info/arhiv-zhurnala/; https://american-issue.info
9. Lakhtin VM, Lakhtin MV, Davydkin VY, Melikhova AV, Davydkin IY, Zhilenkova OG. Enzybiotics with lectin properties and lectinbiotics with enzyme activities - multifunctional modulators of communications in organism: fundamentals and unlimited potential in food industry, biosensors and medicine. *European Journal of Biology and Biotechnology* (EJBIO), 2021; 2(1): 38-46. DOI: http://dx.doi.org/10.24018/ejbio.2021.2.1.150 [Vol 2 | Issue 1 | 22 February 2021] https://ejbio.org/index.php/ejbio/article/view/150; https://www.ejbio.org/index.php/ejbio/issue/archive; https://ejbio.org/index.php/ejbio/issue/archive;

https://ejbio.org/index.php/ejbio/issue/view/7;

10. Lakhtin VM, Lakhtin MV, Aleshkin VA. Innovative aspects of symbiotic lectins and their recognized glycoconjugates. *World Journal of Pharmaceutical Research (WJPR),* December 2023; 12(22): 23-43. DOI: 10.20959/wjpr202322-30275. ISSN 2277–7105. ISO 9001:2015 Certified Journal, www.wjpr.net; https://www.wjpr.net/archive
11. Lakhtin VM, Lakhtin MV, Kapustin IV, Fedorova IM, Blyakher MS, Aleshkin VA. Probiotic lectins and glycoconjugates recognized by lectins: similarity to cytokines, cytokine inducers and immunomodulators. *World Journal of Pharmaceutical Research (WJPR),* 2024; 13(1): 1406-16. DOI: 10.20959/wjpr20241-30791. ISSN 2277–7105. ISO 9001:2015 Certified Journal, www.wjpr.net; https://www.wjpr.net/archive
12. Lakhtin VM, Lakhtin MV, Bairakova AL, Davydkin VY, Aleshkin VA. Bifido-lactobacillar multi-pro/synbiotics: design principles based on ranked properties of strains. *World Journal of Pharmaceutical Research (WJPR),* 2024; 13(13): 9-22. DOI: 10.20959/wjpr202413-31488. ISSN 2277-7105. ISO 9001:2015 Certified Journal, www.wjpr.net; https://www.wjpr.net/archive
13. Lakhtin MV, Aleshkin VA, Kombarova SY. Symbiotic *Gram* positive bacteria: lectin systems and their prospects. *World Journal of Pharmaceutical Research (WJPR),* 2024; 13(13): 23-48. DOI: 10.20959/wjpr202413-31599. ISSN 2277-7105. ISO 9001:2015 Certified Journal www.wjpr.net; https://www.wjpr.net/archive
14. Lakhtin VM, Lakhtin MV, Davydkin VY, Aleshkin AV, Aleshkin VA. Oxidoreductases and lectins recognizing and binding glycoconjugates in biopreparations according to chemiluminescence in a real time. *World Journal of Pharmaceutical Research (WJPR),* 2024; 13(14): 1142-51. DOI: 10.20959/wjpr202414-33177. ISSN 2277-7105. ISO 9001:2015 Certified Journal, www.wjpr.net; https://www.wjpr.net/archive
15. Lakhtin VM, Lakhtin MV, Bairakova AL, Aleshkin VA. The role of the leader strains of some species in the ordering, initiation and switching of subpopulations of microorganisms of other species coexisting in the biotope: prognostic calculation of the series of strains of microorganisms ranked by the ability to biofilm formation in *Lactobacillus-Candida* mixtures. *World Journal of Pharmaceutical Research (WJPR),* 2024; 13; Issue 24: 17-31. DOI: 10.20959/wjpr202421-34891. ISSN 2277–7105. ISO 9001:2015 Certified Journal www.wjpr.net; https://www.wjpr.net/archive

16. *Lakhtin MV, Lakhtin VM, Cherepanova YV, Alyoshkin AV, Krivoruchko EV, Agapova YV, Pospelova VV, Afanasyev SS, Alyoshkin VA. Cultivation of probiotic bacteria in insulin syringes: Regulation and Design.* Materials of the III scientific and practical school-conference "Modern technologies of biological safety". *Edited by G Onishchenko and A Dyatlov. – Protvino (Moscow Region): A-PRINT ZAO, 2011; 281–4. ISBN 978-5-9901795-2-3. (in Russian) https://elibrary.ru/item.asp?id=19502165; http://www.obolensk.org/science/conferences/2011/conference2011.htm*
17. Lakhtin MV, Lakhtin VM, Alyoshkin VA, Afanasyev SS. Synbiotic screening system involving probiotic lectins and synthetic glycopolymers. *Gastroenterology of Saint Petersburg (Saint Petersburg, Russia),* 2016; 3-4: M15-M16. (in Russian). http://gastroforum.ru/?page_id=948; http://gastroforum.ru/wp-content/uploads/2016/12/GSP_2016_3-4_M1-M24.pdf
18. Lakhtin V, Lakhtin M, Aleshkin V. Synbiotic minibioreactor regulating by probiotic lectins, metal cations and glycoconjugates. *In: Proceedings of the 19th Eurocarb (2-6 July 2017, Barcelona).* SCIENTIFIC PROGRAM & ABSTRACT BOOK. Page 591 (Poster 320). EUROCARB 2017 Program, 65: 320. http://www.eurocarb2017.com/images/site/book/LIBRO_COMPLETO_EUROCARB.pdf