INSTITUTE OF MICROBIOLOGY
NATIONAL ACADEMY OF SCIENCES OF BELARUS

INSTYTUT IMMUNOLOGII I TERAPII DOŚWIADCZALNEJ IM. LUDWIKA HIRSZFELDA
L. HIRSZFELD INSTITUTE OF IMMUNOLOGY AND EXPERIMENTAL THERAPY
POLISH ACADEMY OF SCIENCES
POLSKA AKADEMIA NAUK

2nd Forum Science & Technology Days Poland-East

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Bialystok-Bialowieza, Poland

ICT • ECO-ENERGY • HEALTH • NMP • TRANSREGIONAL CO-OPERATION

KAPITAŁ LUDZKI
NARODOWA STRATEGIA SPÓJNOŚCI

UNIA EUROPEJSKA
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FUNDUSZ SPOŁECZNY

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Name of Institution:

Institute of Microbiology

INSTITUTE OF MICROBIOLOGY
NATIONAL ACADEMY OF SCIENCES OF BELARUS

Department ***Belarus Collection of Microorganisms***

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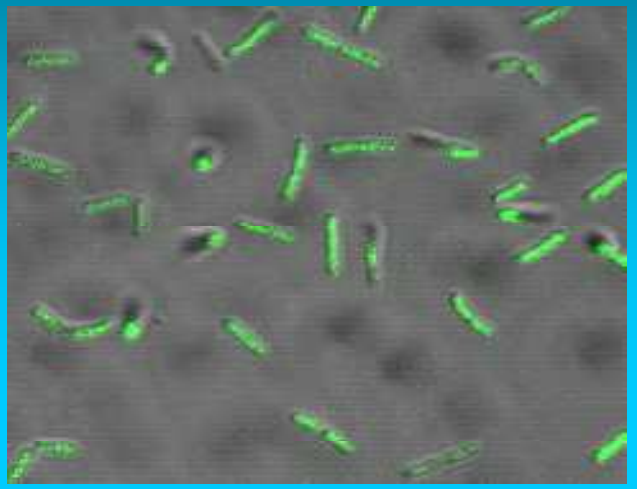
Institution profile

Institute of Microbiology is active in development and patenting the documentation packages for preventive therapeutic preparations, i.e. Enterobifidin, Bactryl, Bifilac, Bifidobacter and curative dietetic product Bifibac.



Institute is certified as the Biotechnological Centre that combines research and pilot-plant production of various products (food additives, bioactive supplements, probiotics, microbial fertilizers and plant biological control agents) with marketing activity to promote sales of microbial products.

Collection of microorganisms, including bifido- and lactic acid bacteria, was entered by ordinance of the Council of Ministers of Belarus Republic into State Register of scientific objects estimated as National asset under formal designation Belarusian Collection of non-pathogenic microorganisms (Collection of type and valuable industrial non-pathogenic microbial cultures, Institute of microbiology BNAS)

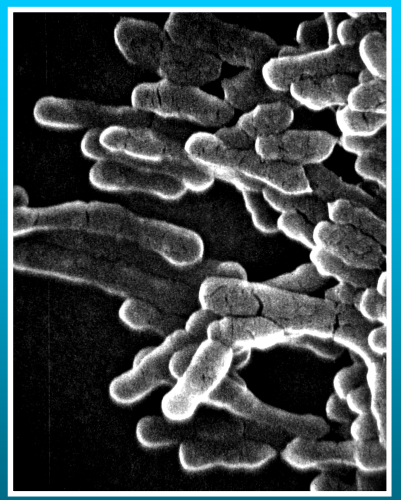
The Lab took participation in international research projects, such as:

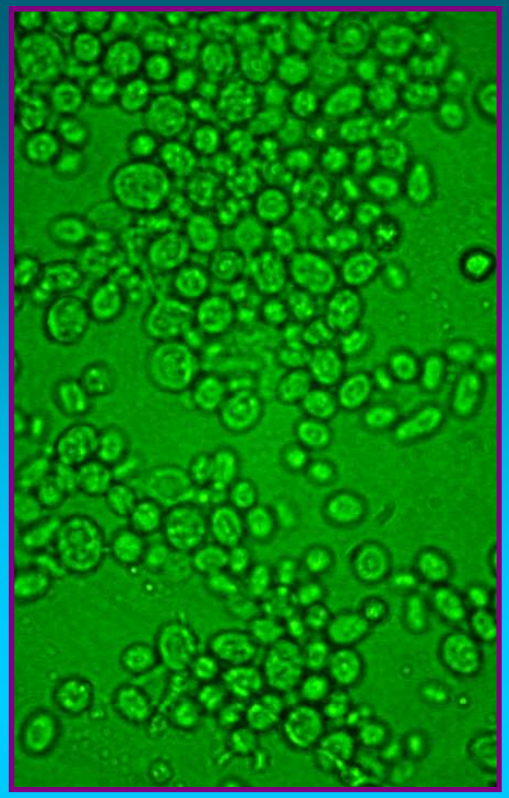
- ✓ *"Maintenance, study and development of Culture Collection of microorganisms in Armenia, Ukraine and Belarus" (the INTAS grant 93-3512)*
- ✓ *«Bacterial glycoconjugates in modern healthcare - new extraction approach for immunostimulants and probiotics» (VISBY-01720/2004)*
- ✓ *«Glycoconjugates from Bifidobacterium in prevention and treatment of celiac disease» (VISBY - 01147/2007)*
- ✓ *"Freezing of microorganisms - objects of new biotechnologies" funded by General Secretariat of Funds of basic Research of Belarus and Ukraine (the grant B07K-024)*
- ✓ *«Research of antigens of microorganisms – probiotics» funded by General Secretariat of Funds of basic Research of Belarus and Russian Federation (the grant X08P-072/2008)*

Major trends of collection activities are:

- *setting up the collection stock of microbial cultures valuable for various areas of microbiology and biotechnology;*

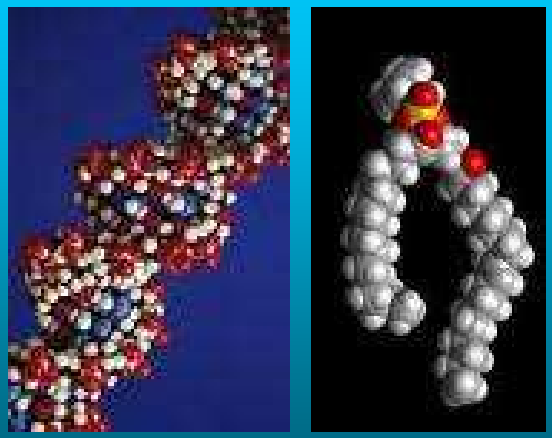
- *replenishing of the fund with type and reference microbial strains, identification of bacteria, filamentous fungi and yeasts, studies on physiology of microorganisms affiliated to different taxonomic groups;*
- *maintaining viability of deposited microorganisms by long-term conservation techniques;*
- *classifying of information on collection stock cultures summed up in the respective data bank, providing expertise on subjects related to identification, depositing of microbial strains for patenting procedure, supplying cultures upon request of the customers.*



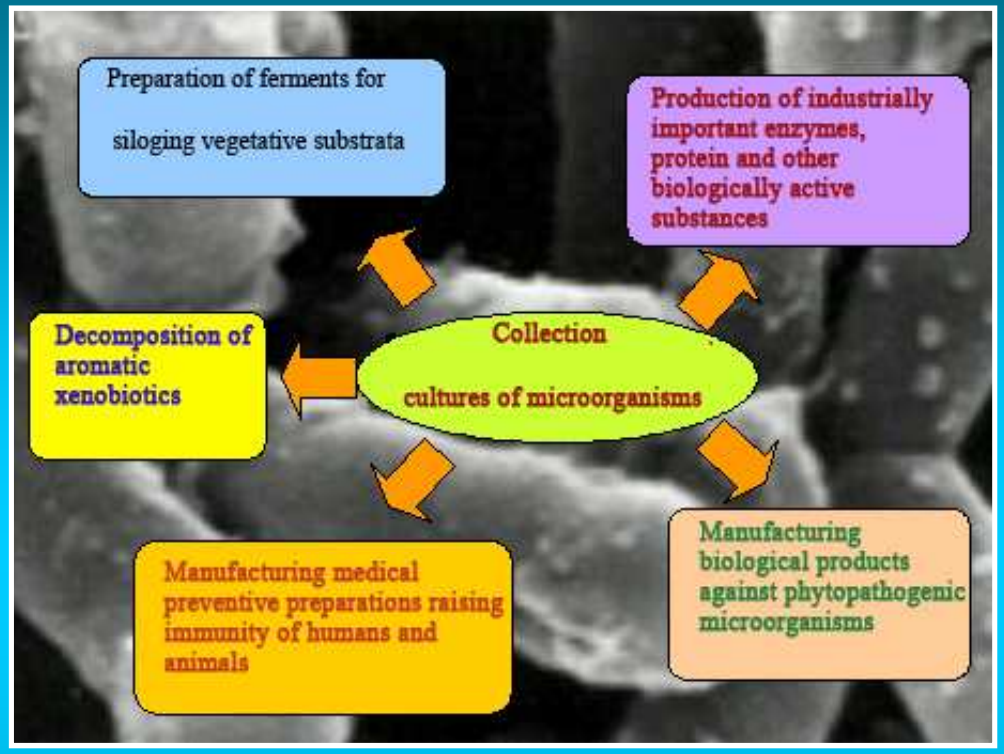


Basic guidelines for the activities of Belarusian collection of non-pathogenic microorganisms are isolation of new microbial strains from natural sources which are potential objects for industrial, agricultural and environmental biotechnologies, compiling data bank characterizing properties of bacteria, filamentous fungi, yeasts and bacteriophages, elaboration of theoretical and practical recommendations for application of industrial strains from collection stock

A promising trend in functioning of the collection is introduction of biochemical methods for detection of phospholipids and glycolipids as chemotaxonomic markers and molecular-genetic methods of identification of microorganisms based on 16S ribosomal RNA gene sequences



Valuable industrial microbial strains could be used in:



❖ *manufacturing enzyme preparations and ferments ensiling plant substrates;*

❖ *producing preventive-therapeutic compositions enhancing immune potential in humans and animals;*

❖ *development of biological agents to control plant pathogens;*

❖ *bacterial preparations for degradation of toxic organic substances and bioremediation of natural and industrial media*

Dr. Galina Novik

- the Head of the Belarusian Collection of microorganisms

- *over 160 scientific papers*
- *5 National patents*
- *was the team leader in scientific collaboration with the international projects with the IITD PAN, Lund University, Institutes of Russian and Ukraine Academies of Sciences*.

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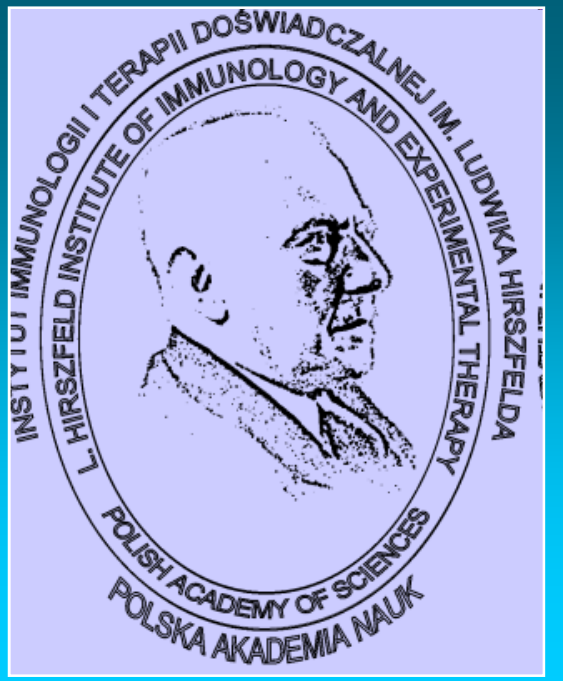

Prof. Andrzej Gamian, PhD
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Belarus team has an expertise in isolation and identification of probiotic bacteria producing bioactive compounds (enzymes, organic acids, extracellular proteins and polysaccharides). Methodology mastered by the team leader and members: isolation, purification and assay of biological activity of protein-polysaccharide complexes, glyco- and phospholipids of probiotic microorganisms and microbial glycoconjugate structure impact on host immune response

Collaborators

Anatol Gordienko[1]
Elena Kiseleva[2]
Estera Szwajcer Dey[3]
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[2] *Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich 5, 220141 Minsk, Belarus*
[3] *Lund University, Pure and Applied Biochemistry, Box 124, 212 00 Lund,*

Information on project

Title:

Biologically active compounds of probiotic bacteria and their use in biotechnology and medicine

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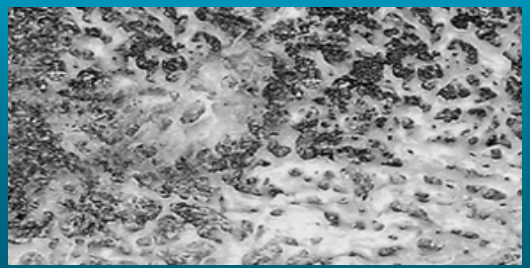
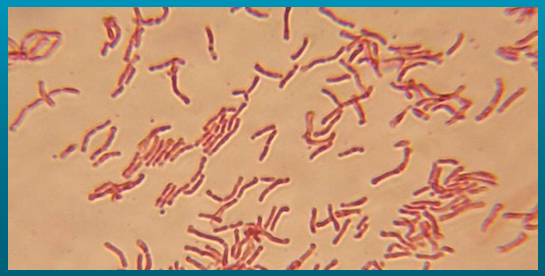
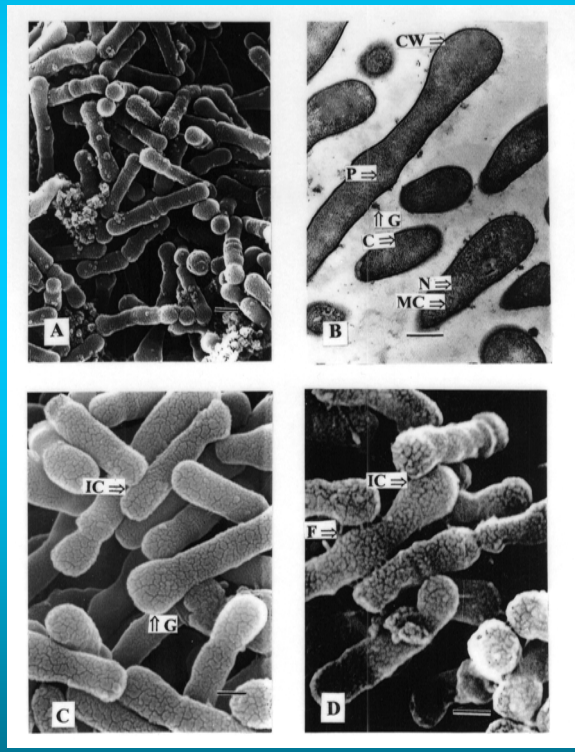
Description:

Major attention is focused on development of bioactive preparations and biomaterials on the basis of cells and cellular components, mainly glycoconjugates, of probiotic microorganisms. Research is centered on production of a new class preparations and biomaterials for medicine possessing high biological activity.

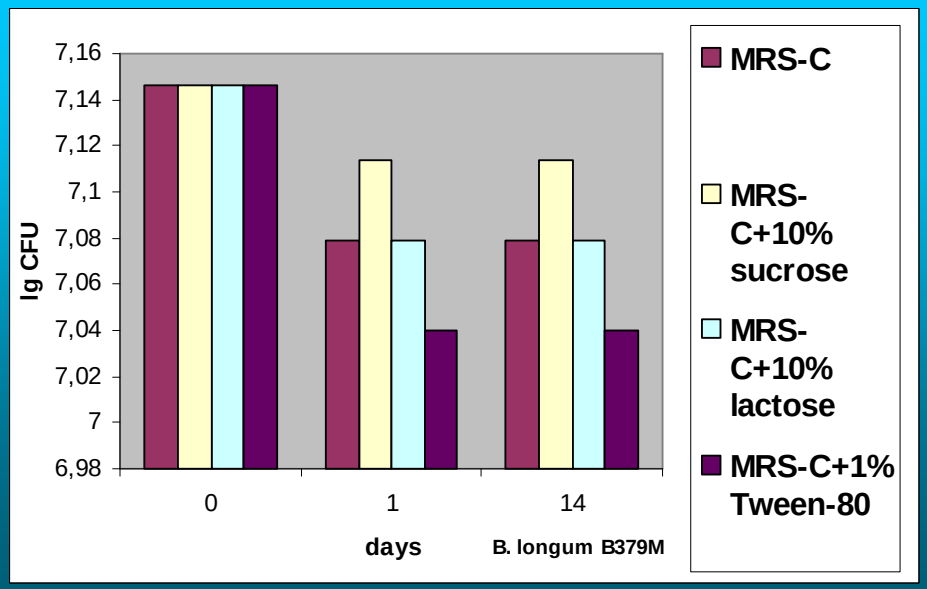


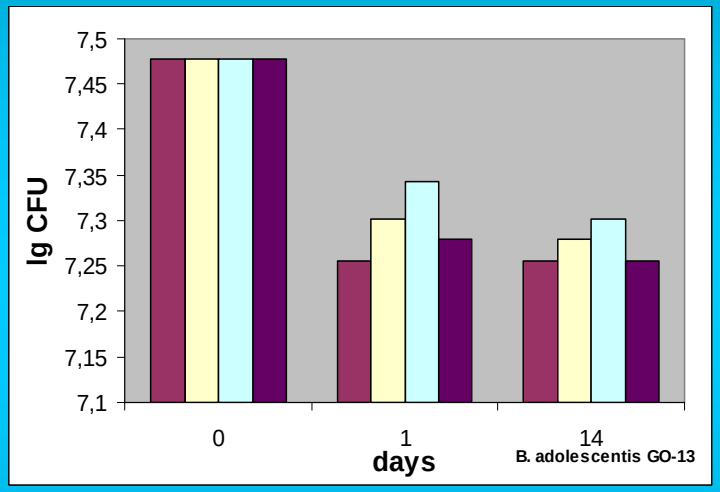


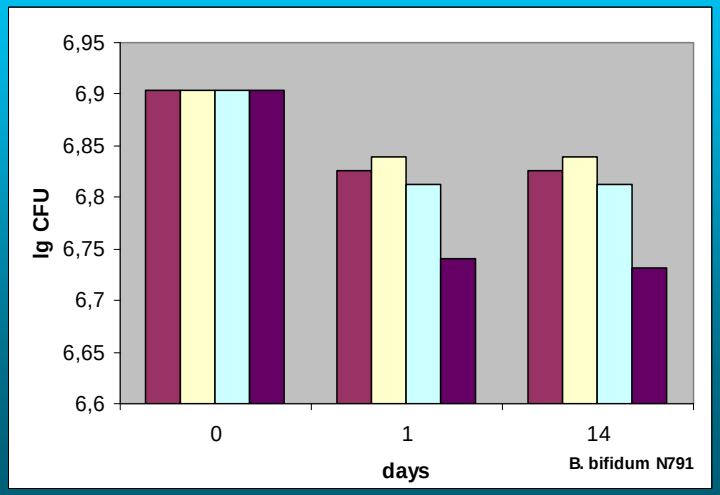


The effect of different cryoprotective additives: sucrose (10%), lactose (10%), Tween-80 (1%) on survival and retention of acidification activity of bifidobacteria was examined. No significant differences ($p<0.05$) in viability of bifidobacteria after rapid cryopreservation with and without cryoprotectors were observed. Media traditionally used for the cultivation of bifidobacteria proved to have good cryoprotective properties and can be used for preservation of these microorganisms without additional protectors.

Viability of Bifidobacteria after freezing with different cryoprotectors



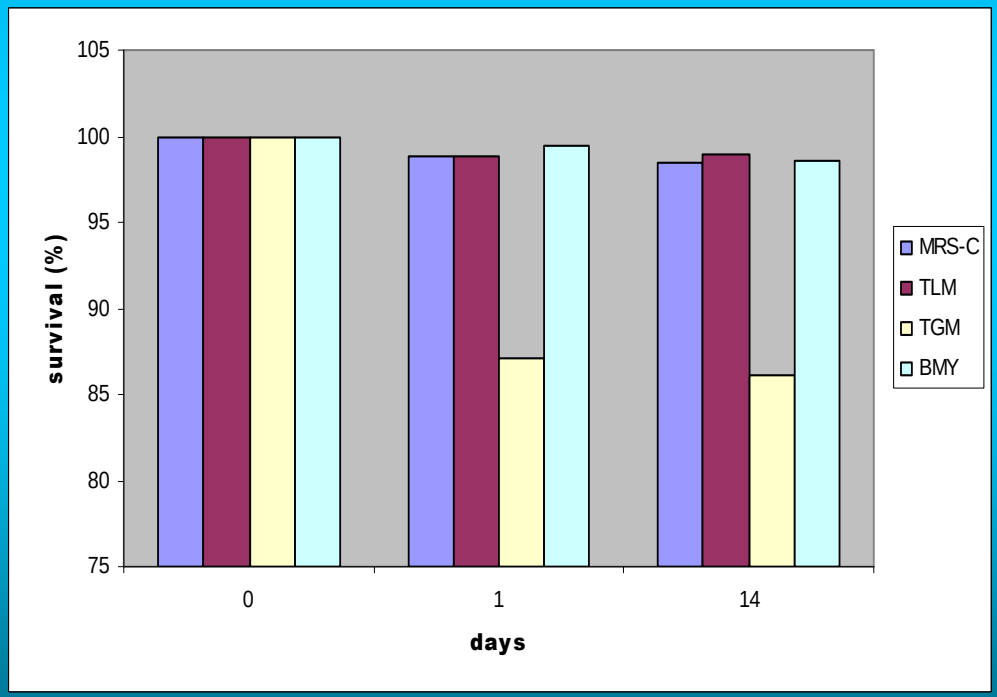




Growth activity of B. adolescentis 94 BIM in milk after freezing in different media

Medium	before freezing		1 day after freezing		14 days after freezing	
	time (h)	pH	time (h)	pH	time (h)	pH
MRSC	18	4,29	20	4,60	20	4,56
TGM	18	4,37	-	5,23	-	5,52
TLM	18	4,18	18	4,36	18	4,40
BMY	18	4,20	20	4,52	20	4,58

The effect of composition of growth and freezing media on viability and stability of metabolic activity of bifidobacteria during cryopreservation with rapid cooling kinetics was examined. Four different media for bifidobacteria cultivation, namely MRS, supplemented with 0.05 g/l L-cysteine (MRS-C), tryptone-lactose (TLM), "Bifidobacterium-", supplemented with 0.5% yeast extract (BMY) and thioglycolic (TGM), were tested. Trypton-lactose medium proved to be the best for bifidobacteria cryopreservation.



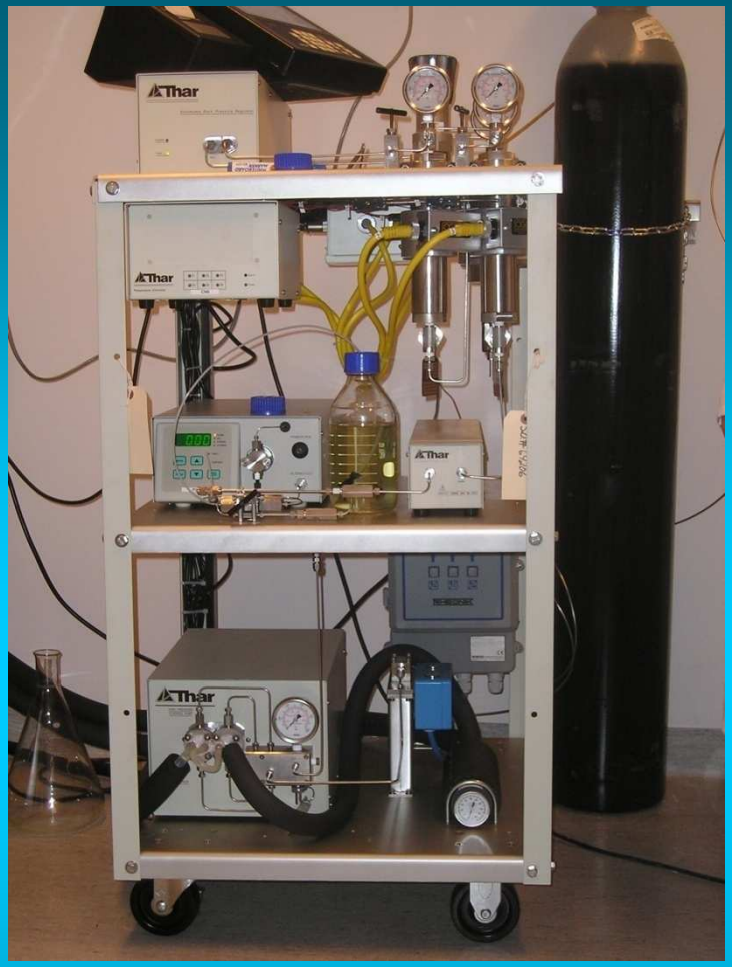
Survival of *B. adolescentis* 94 BIM after cryopreservation in different media

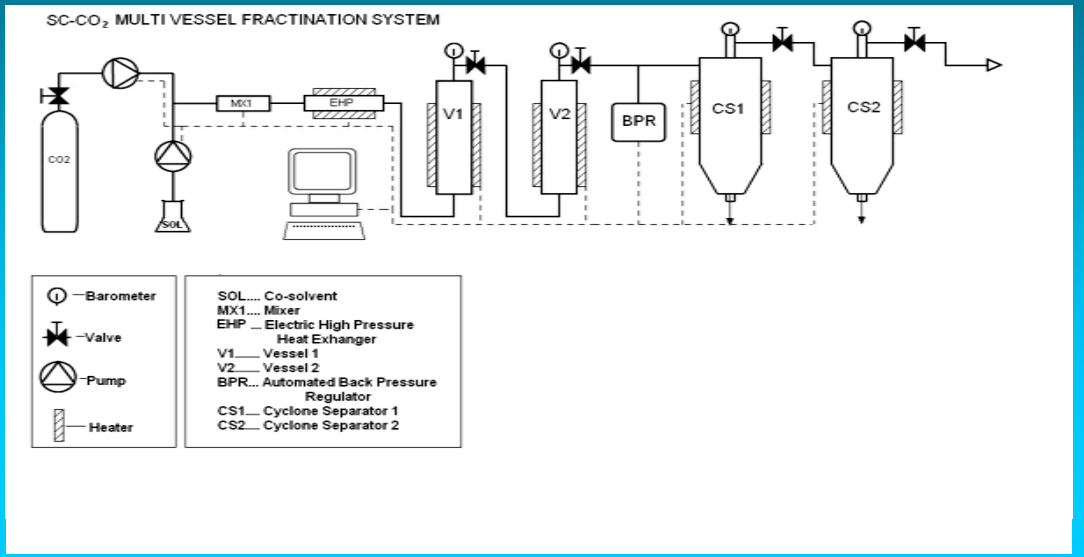
A novel procedure for the isolation of glycolipids from bifidobacteria using supercritical carbon dioxide

Reid has stated that more detailed research is necessary to understand which reactive compounds are obtained from probiotic bacteria, how they act e. g. on the immune system, and what are their mechanisms of action (2003). One of the limiting factors has been methodology for isolation of pure compounds such as polysaccharides and glycolipids. Classical methods of purification are very inefficient, laborious, produce toxic waste and give low yields.

We have therefore developed an improved method for extraction and purification of glycolipids, so that their immunological properties can be studied. If beneficial effects are discovered it could provide material for the production of adjuvants to be used in modern health care.

Supercritical carbon dioxide (scCO2) extraction is simple, rapid, non-flammable, also inexpensive and environmentally friendly. The solvent capacity of supercritical carbon dioxide is mainly a function of density and this in turn can easily be changed by varying pressure and temperature or by addition of small amounts of co-solvents (Francisco and Dey, 2003).





Schematic diagram of the scCO2 extraction system

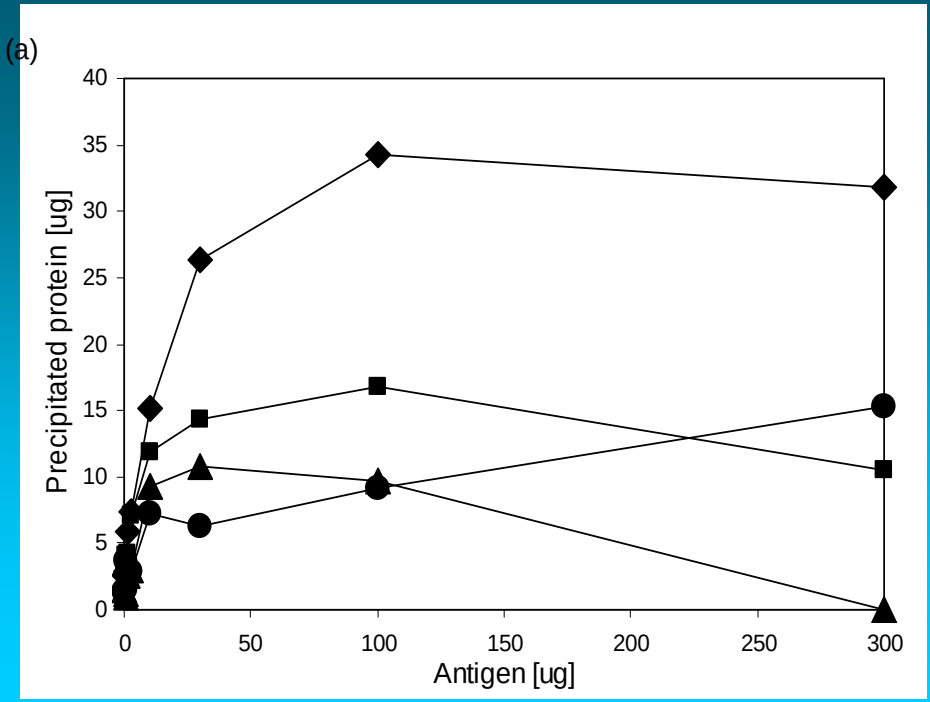
Only the extraction vessel 2 (V2) and cyclone separator 2 (CS2) of 50 ml volume, were used.

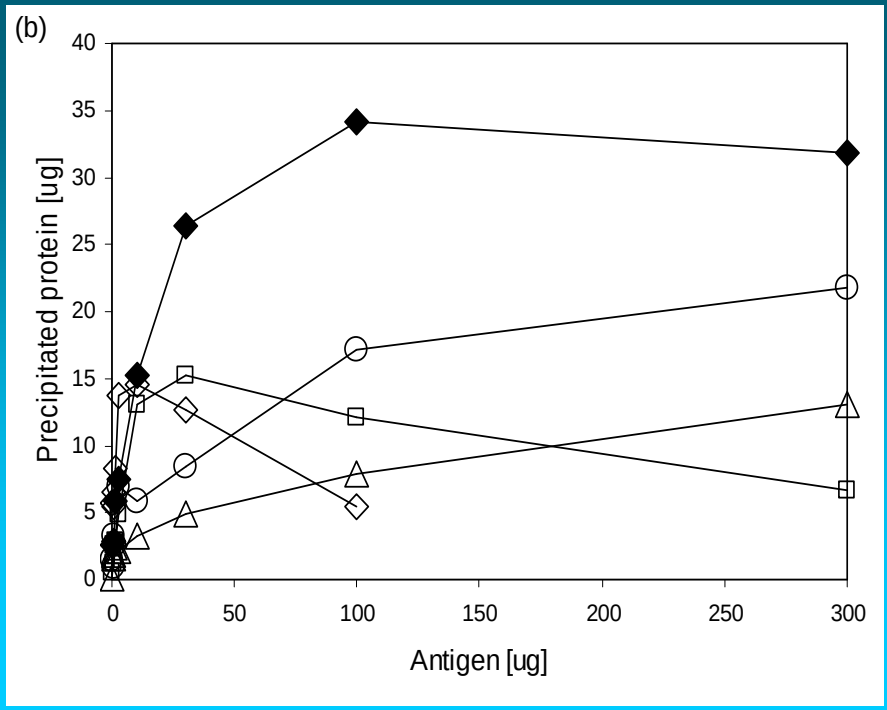
Preparation	Component (molar ratio)		
	Glucose	Galactose	Mannose
PS-CW-EL	1.0	0.3	-
F_1	1.0	-	-
F_2	1.0	1.1	-
PS-CW-EM	1.0	0.9	-
F_1	1.0	-	-
F_2	1.0	1.7	-
PS-CW –TSB	1.0	0.2	-
F_1	1.0	-	-
F_2	1.0	0.8	-
PS-CW-CLY	1.0	0.1	-
F_1	1.0	-	-
F_2	1.0	0.3	-
PS-SN-EL	1.0	0.3	-
F_1	1.0	-	-
F_2	1.0	1.6	0.1
PS-SN-EM	1.0	1.2	1.1
F_1	1.0	0.2	3.7
F_2	1.0	2.0	5.0
PS-SN-TSB	1.0	0.7	0.1
F_1	1.0	0.4	0.1
F_2	1.0	0.9	0.1
PS-SN-CLY	1.0	-	20.5
F_1	1.0	0.9	10.4
F_2	1.0	0.8	15.4

Sugar analysis of polysaccharides isolated from *B. adolescentis* 94-BIM cell mass (PS-CW) and culture supernatants (PS-SN) before and after separation on DEAE-Sephadex A-25 column (F1 and F2)

Abbreviations: EL, Eagle medium with lactose; EM, Eagle medium with mannose; CLY, casamino acids with lactose and yeast extract medium; TSB, tryptic soy broth; –, component not detected

The glucan type of polysaccharide was produced in different cultural media, including synthetic medium supplemented with lactose or mannose. This polysaccharide was also found in PS preparations isolated from cell-free supernatants after cultivation of bacteria in EL and TSB media. In case of PS-SN-EM and PS-SN-CLY preparations, the main constituent was mannose.

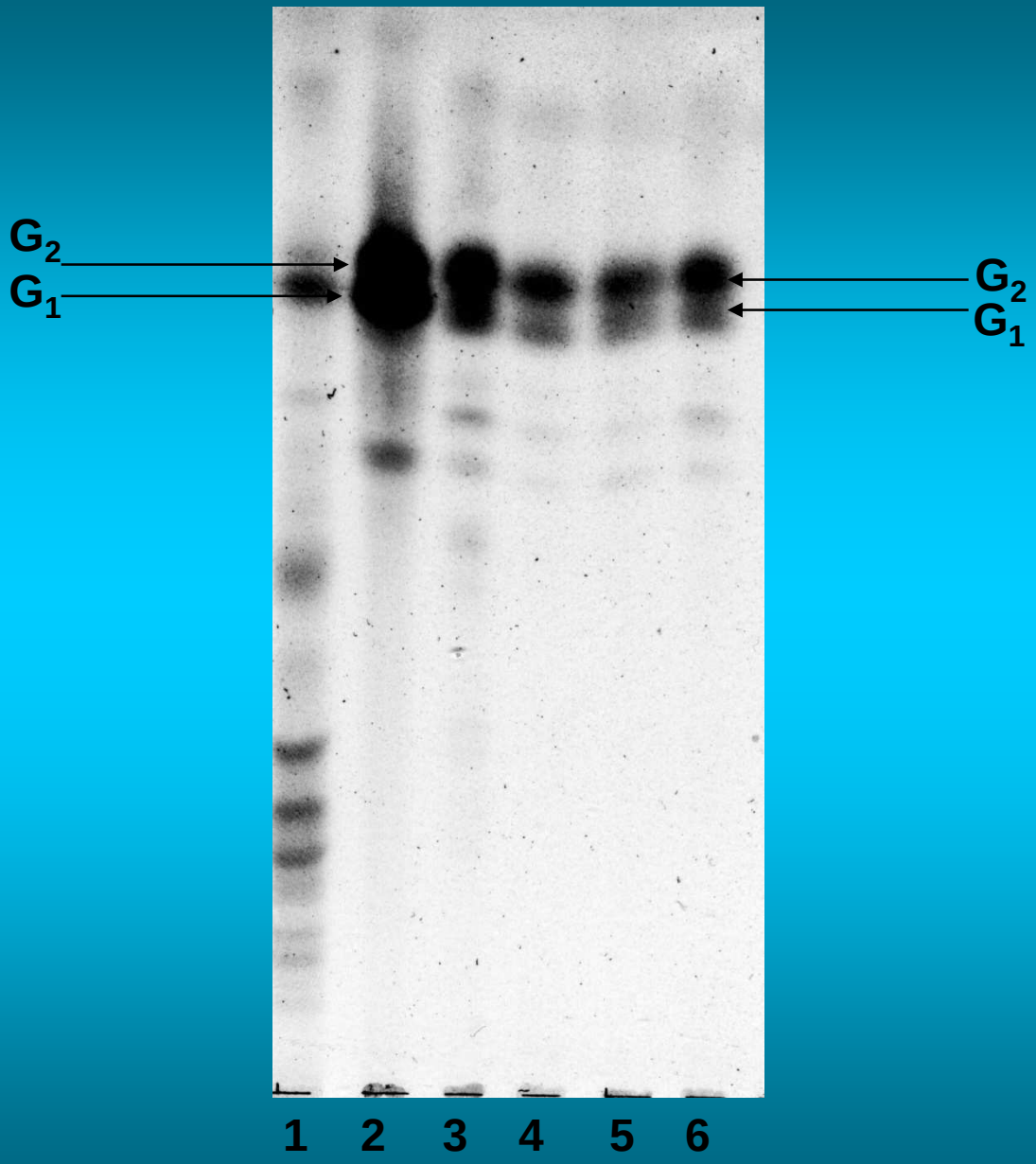




Quantitative immunoprecipitation of the rabbit serum anti-*B. adolescentis* 94-BIM cells grown in EL medium with polysaccharide fractions isolated from bacterial cell mass (a) and from culture supernatant (b). ◆, PS-CW-EL; ■, PS-CW-EM; ▲, PS-CW-TSB; ●, PS-CW-CLY; ◆, PS-SN-EL; □, PS-SN-EM; Δ, PS-SN-TSB; ○, PS-SN-CLY

Abbreviations: PS, polysaccharide; CW, cell wall; SN, supernatant; EL, Eagle medium with lactose; EM, Eagle medium with mannose; CLY, casamino acids with lactose and yeast extract medium; TSB, tryptic soy broth

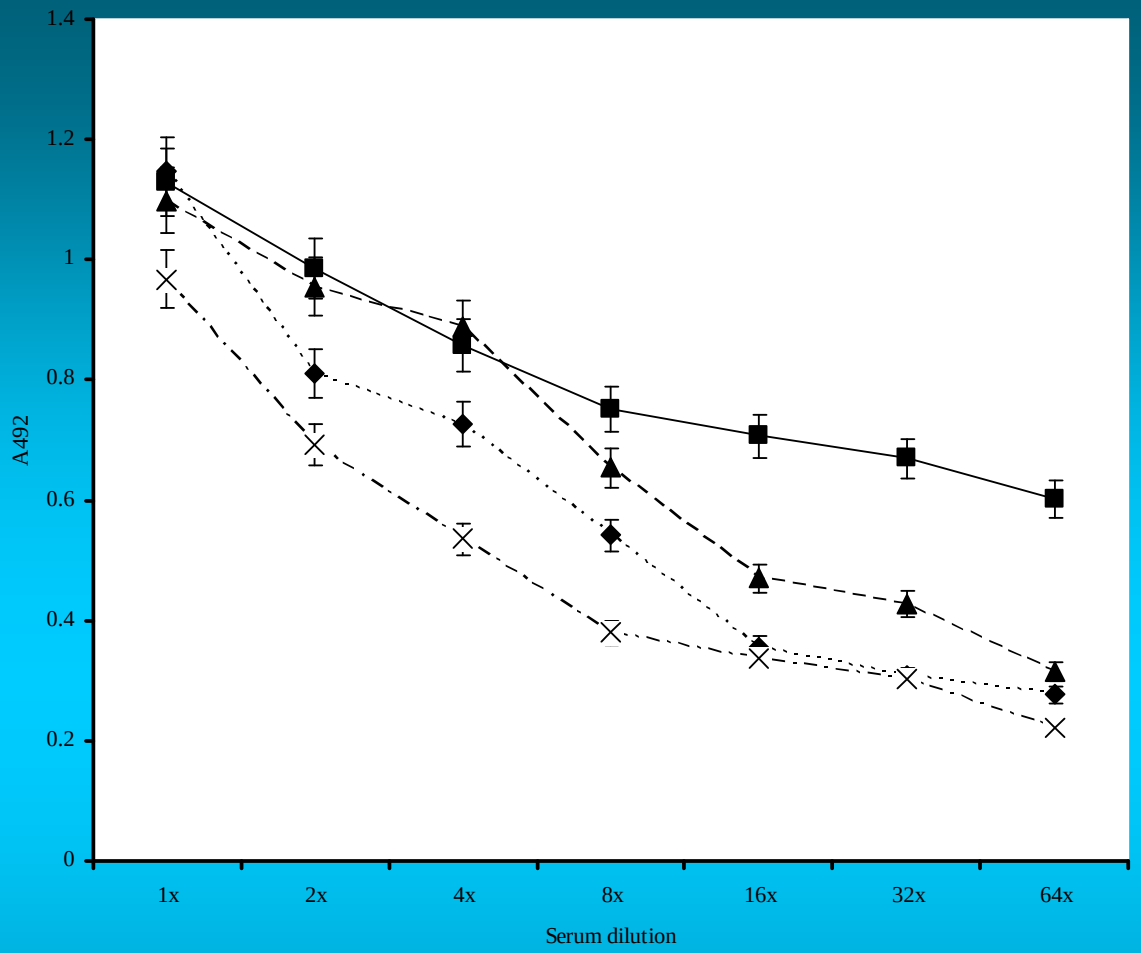
Immune rabbit serum against whole bacterial cells reacted predominantly with homologous polysaccharide but also with polysaccharides isolated from cells cultured in different media. The facts that cell surface PS from EL medium was more densely branched (ratio of 4-substituted to 4,6-disubstituted Glc residues was 1:1) and more reactive with homologous serum than PS from other media (4-substituted to 4,6-disubstituted Glc residues in ratio 3:1) might be of biological relevance.



Glycolipids isolated by modified scCO2 compared to the organic solvents isolation method. Lane 1, partially purified lipid, lane 2, reference glycolipids, lanes 3, 4, 5 and 6, fractions isolated by modified scCO2

Abbreviations: G1 and G2: major glycolipids.

By employing the new modification of scCO2, the major glycolipids of bifodobacteria can be very effectively extracted. Sixty mg of glycolipids were isolated from 1g of freeze-dried biomass (6% of yield). TLC analysis of different scCO2 fractions also showed predominance of major glycolipids. Similar glycolipids (G1 and G2) were found in the partially purified lipid and in the reference fractions.

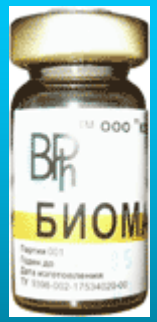


ELISA analysis of isolated glycolipids, using scCO2. Anti-serum was titrated against reference glycolipids (__×__), the scCO2 fractions (...◆..., __■__, ---▲----), obtained at different temperatures.

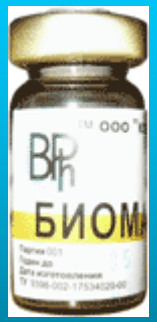
Enzyme linked immunosorbent assay showed that all glycolipid antigens extracted by this scCO2 method possess a high level of immunological reactivity. This procedure does not change the natural structure of this compound. The new method of glycolipid isolation can be scaled up to industrial size.

RESEARCH STRATEGY OF DESIGNING MEDICAL MATERIALS BASED ON TITANIC ALLOYS WITH BIOLOGICALLY ACTIVE COATING

Modern achievements in development of new biomaterials have been scaled up from experimental level to clinical surgery practice. Biomatrix represents the complex of biologically active substances specially elaborated to ensure effective implant integration with body tissues. The vital criteria of Biomatrix efficiency are absence of cell toxicity, promotion of adhesion and fixation, proliferation and differentiation of surface cells, lack of inflammatory reaction to biomaterial and immune response, evacuation by common metabolic pathways. Biomatrixes are applied for engineering muscular, epithelial and bone tissues; they contain optimal ratios of adhesive proteins, like collagen, and glycosaminoglycans , growth factors and other biologically active substances supporting proliferation and differentiation of tissue cells.



«Biomatrix - implant»
an osteoinductive material



«Bioimplant»
an anti-inflammatory
osteoinductive material

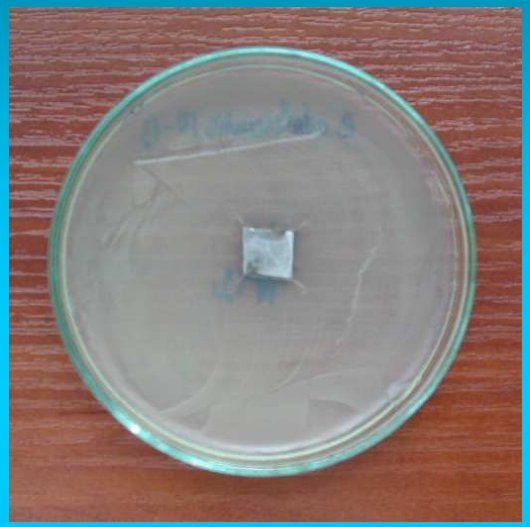
«Allomatrix - implant»
an osteoinductive material based on allocollagen

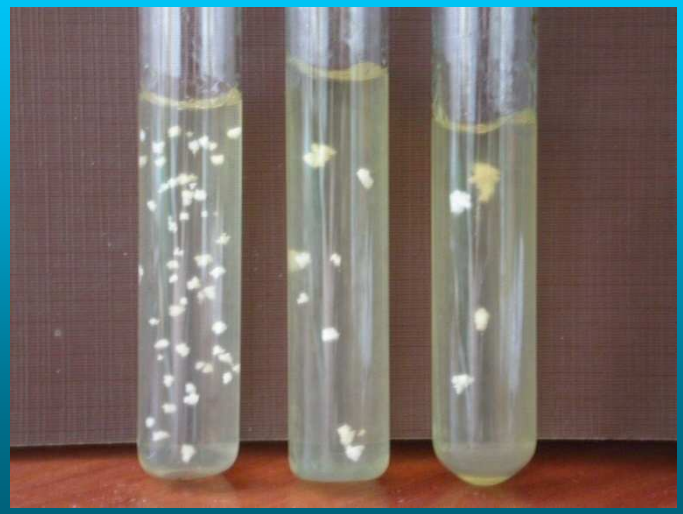
Biomaterials are manufactured in Russia. The form of products: pellets, strips, gels, disks, blocks.

Research on biocompatibility of medical titanic alloys and probiotic microorganisms of genus *Bifidobacterium*

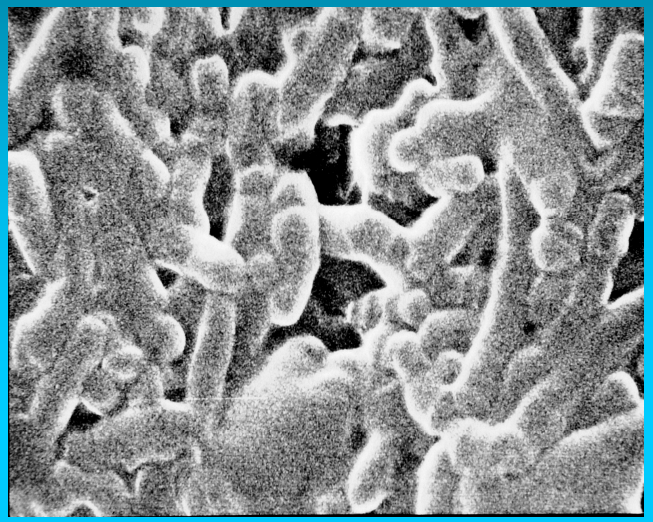
in vitro experiments

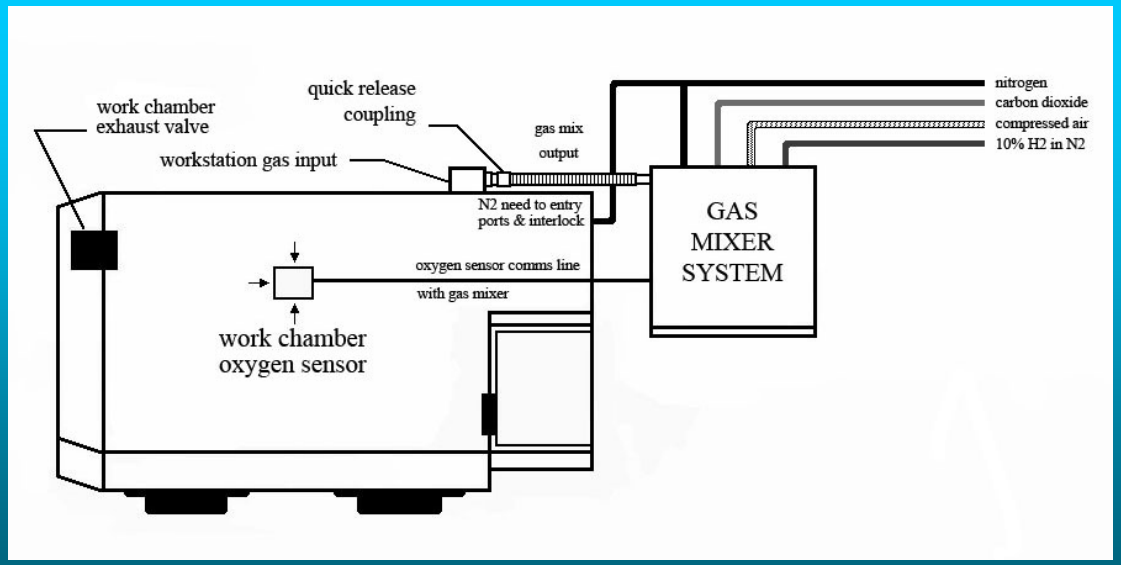
Absence of nonspecific toxicity of studied titanic alloys, their biocompatibility with probiotic microorganisms of genus *Bifidobacterium,* and high cell adhesion capacity on the surface of tested materials was established.

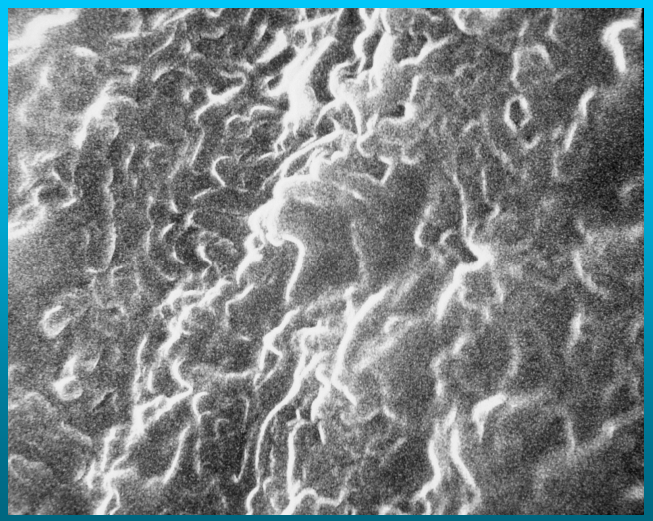




Application of biologically active coating by using anaerobic station BUG BOX M







Components of bifidobacteria influence formation of immune complexes TPO-AAB

Bifidobacterium adolescentis 94 BIM

supernatant

biomass

Sonication

Centrifugation (36 000 g)

supernatant — *sediment*

preparation 1

+sodium cholate

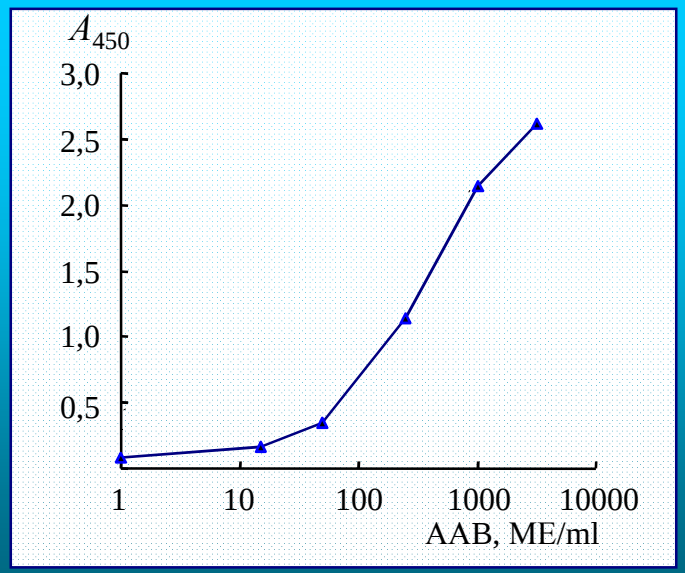
supernatant — *sediment*

preparation 2

+triton X-100

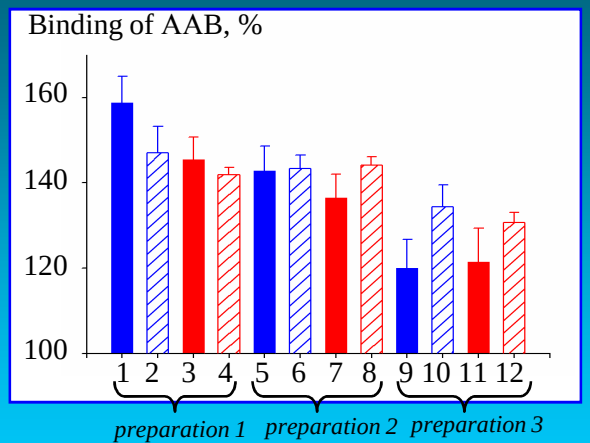
supernatant

preparation 3

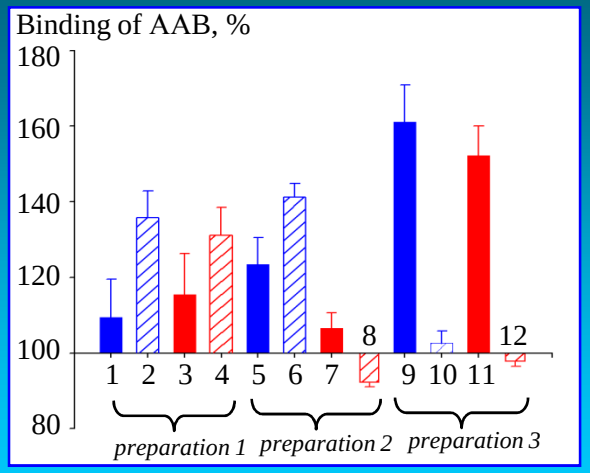


The scheme and calibrating graph of immune enzyme assay of autoantibodies to TPO

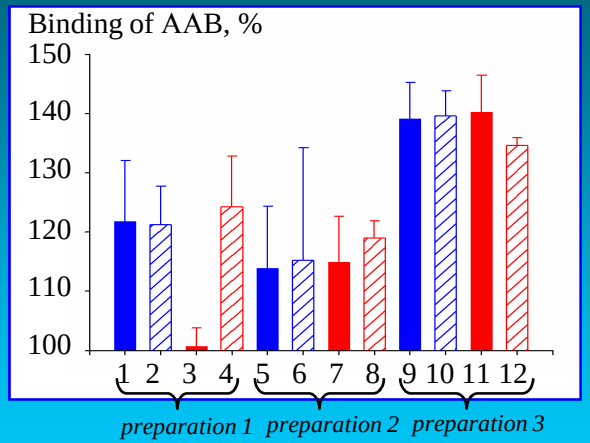
Interaction TPO with AAB in presence of preparations 1, 2, 3



Incubation of TPO with preparations 1, 2, 3 before immobilization



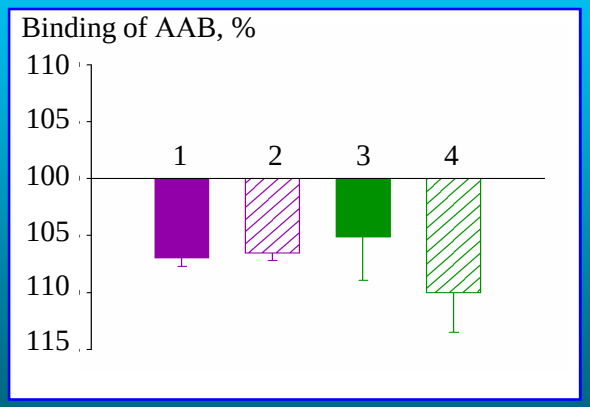
Incubation of TPO with preparations 1, 2, 3 after immobilization



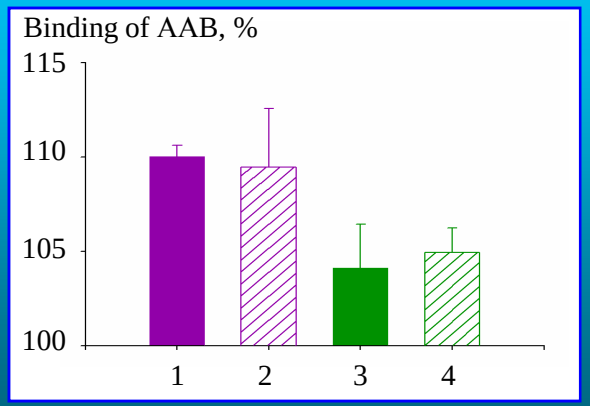
Bifidobacterium bifidum
-- // -- // -- // -- (1/10)

B. adolescentis 94 BIM
-- // -- // -- // -- (1/10)

Interraction of TPO with AAB in presence of bacterial polysaccharides



Incubation of TPO with bacterial polysaccharides before immobilization



glucose *mannose* *polyglucose* *polymannose*

Preparation 1 – secretory polysaccharides and cytosol.

Preparations 2 and 3 - bacterial membrane components solubilized with sodiim cholate (2) and triton X-100 (3)

Control tests did not contain components of microorganisms at any stages of the analysis.

Significant deviations from the control exceeding 15 %

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