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Original article

USE OF HIGHLY DISPERSED SILICA IN BIOTECHNOLOGY OF COMPLEX PROBIOTIC PRODUCT BASED ON BIFIDOBACTERIA

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Background. The probiotics immobilization technology is one of the most effective ways for controlled and continuous delivery of viable cells into the intestine. It is well known that multifaceted physiological roles of bifidobacteria are to normalize and stabilize the microbiocenosis, to form intestine colonization resistance, to synthesis amino acids, proteins and vitamins, to maintain non-specific resistance of the organism and so all. Such a wide range of positive effects on the macroorganism allows us to consider bifidobacteria as a basis for functional immobilized healthcare products development.
Objective. Taxonomic position determination of the *Bifidobacterium longum* strain selected for immobilization, study of the viability of this bifidobacteria strain in a complex probionic product based on highly dispersed silica in simulated gastrointestinal tract's conditions and after freeze-drying.
Methods. The production strain *Bifidobacterium longum* IMV B-7165 from the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine collection of industrial strains has been used in the study. It was isolated from the healthy human infant's gastrointestinal tract.
Commonly used bioinformatics, microbiological, biotechnological and statistical methods have been applied.
Results. The best alignments for the sequence of bifidobacteria isolate "4202" 16S rRNA (it was previously deposited as *Bifidobacterium longum* IMV B-7165) and classic dendrograms based on these results were performed. According to the results of microscopic studies of samples of microorganisms with highly dispersed silica products ("Enterosgel", "Sillard P" and "Toxin.Net") it was found that the immobilization of the *Streptococcus thermophilus* and bifidobacteria cultures did not differ fundamentally. To study the immobilization effect on the bifidobacteria preservation and properties the following carriers were used: "Enterosgel", "Toxin.NET" and "Sillard P". The survival of immobilized bifidobacteria was further studied in simulated gastrointestinal conditions: immobilized cells are better protected from acid and bile, although with increasing acidity, survival decreases in both control and immobilized cells.
Conclusions. The taxonomic position of a bifidobacterial isolate from the healthy human infants used in immobilization studies was clarificated (*Bifidobacterium animalis subsp lactis*). Under the simulated conditions of the upper gastrointestinal tract in the case of acid and bile impact, the best survival was demonstrated by immobilized cultures of bifidobacteria together with the Enterosgel sorbent (a content of 10% by weight of the culture). The survival of immobilized preparations after freeze-drying was slightly reduced in the case of immobilization on the "Enterosgel" and "Toxin.NET" samples of enterosorbents (a content from 15% to 25% by weight of the culture). The best results were observed in the case of immobilization of bifidobacteria with 5% content of the "Toxin.NET" enterosorbent (enterosgel + inulin).

Keywords: bifidobacteria; highly dispersed silica; taxonomic position; immobilization; healthcare products.

Introduction

The intestinal microbiota has a number of important functions in the life of the human body: protective, digestive, metabolic and immunomodulatory. The use of probiotic for the normal microbiota of the gastrointestinal tract (GIT) maintenance is a useful method of correction. But unfortunately, as it is well known only tenth of all probiotic microorganisms attach to the intestinal wall [1, 2]. The levels of bifidobacteria and lactobacilli in the intestine are the indicators of the organism's microecological status. Therefore, controlled delivery of living cells (as well as their duodenal transplantation) and their release into the gastrointestinal tract is a promising area for studies [3]. One type of such delivery is the use of carrier matrices that bind to the target component and prevent its premature release or destruction. Such materials can be activated carbon, lignin, chitin, cellulose, sodium alginate gel, silicon dioxide, aluminium oxide with carbon film, and others. It is known that complex preparations of probiotic cultures, immobilized on carriers, better tolerate both conditions during drying (lyophilized or spray dryer) and realised in the gastrointestinal tract [4].

It has been shown that some methods of probiotic bacteria microencapsulation, in particular in alginate gel, are able to prevent their damage in fermented milk products, improve their viability and maintain a high level of therapeutic activity during the usage period [5]. Other variants of encapsulated materials such as anhydrous milk fat are also investigated [6].

Sorbex (activated charcoal) immobilized and frozen probiotics promoted a faster colon microbiota normalization of rats with chemotherapeutic dysbiosis and elimination of pathogenic microbiota in comparison with the action of native probiotics, enterosorbents and probiotic-sorbent mixtures.

The efficacy of *Lactobacillus rhamnosus* encapsulation into mesoporous alginate-silica with modeling the existence of cells in the gastrointestinal tract was also demonstrated. The viability of microorganisms increased with the addition of buffer. Mesoporosity (50 nm) of silica promoted the nutrient metabolites higher permeability and, consequently, higher cell growth without their release [8]. Some attempts have been made to cultivate probiotic strains with the addition of silica. In particular, it was shown that the addition of 2% "Aerosil A300" to the culture medium promoted biomass increase up to 40–75%, with simultaneous acidity increasing, while antagonistic activity of *L. plantarum* and *B. adolescentis*, *L. lactis* probiotic strains did not change significantly [9].

Highly dispersed silica ("Enterosgel", "Atoxyl", "Sylix", "Aquasil", etc.) is approved for use as enterosorbent, in poisoning treatment to reduce intoxication, as a component of some ointments [10]. Dispersed silica is of interest due to its biocompatibility, safety and high adsorption properties (mainly due to silanol groups). Recently, interest in mesoporous silicas is growing again due to their possible use for the development of drugs targeted delivery means, implants' antibiotic film coatings, tumors diagnostics methods using fluorescent properties of Cornelldots (C-dots) [11].

Scientific publications data allows linking the biological consequences of cell-silica interactions with such parameters as particle shape and size, surface area, number of silanol groups, degree of hydrophilicity, crystallinity index, presence of atoms of other elements, surface ability to generate oxygen radicals and selective adsorption of biologically significant molecules.

The long-term study of highly dispersed silica in different doses did not reveal any significant toxicity. But it was noted that crystalline silica physicochemical modification, changing particle size and surface, could significantly increase biocompatibility [12]. The interaction between highly dispersed silica and cells has been studied in view of possible nanoparticles toxicity at different ways of administration: inhalation, intradermal penetration, conjunctival instillation and parenteral penetration. Experiments have shown a highly safety of this compound (class IV of toxicity) [13].

Therefore, there are reasonable assumptions to suppose that highly dispersed silica can be a carrier for a complex probiotic healthcare products with prolonged action throughout the entire gastrointestinal tract.

There are a number of technological problems in the complex probiotic products development: silica optimal forms selection (nano-, highly dispersed, mesoporous, etc.) and their ratios with probiotic cultures, possible need to modify the silica surface, choice of product procedures, buffers, drying and storage regimens, etc.

Previously, the properties of complex products of probiotic monocultures *Streptococcus thermophilus* IMV-7249, *Bifidobacterium longum* IMV 7033, immobilized on xerogel "Sillard P" and modified hydrogel "Enterosgel" were studied. The best survival results were shown for *S. thermophilus*. Bifidobacteria of the selected strain were more sensitive to the hemolytic action of highly dispersed silica [14]. Bifidobacteria inhabit newborns' intestines during breastfeeding (from mother's milk) and, entering into a stable symbiosis with these tissues, accompany the person throughout life, causing a beneficial effect on health [15]. It is bifidobacteria that play a significant role in the normal development of microbiota in the human infants, preventing dysbiosis, restoring normal microflora after candidiasis, reducing the ability of the intestine to absorb amines, which increase blood pressure in the elderly. These and other useful properties of bifidobacteria determine the desire to include it into complex healthcare product immobilized on silica enterosorbents.

Among antimicrobial, immunomodulatory, anti-inflammatory and antiviral drugs with native bifidobacteria cells at present such metabolic drugs design involves the development of medicines containing biologically active metabolic products and separate structural components of probiotic cells [16], as even cells fragments are considered useful in terms of their positive effect on the body.

Therefore, the development of effective drugs based on probiotic strains of bifidobacteria for the formation of beneficial intestinal microbiota and microbiome therapy is an urgent task.

The *aim* of the study: taxonomic position determination of the *Bifidobacterium longum* strain selected for immobilization, study of the viability of a bifidobacteria and highly dispersed silica complex healthcare products in simulated gastrointestinal tract's conditions and after freeze-drying.

Materials and methods

The production strain *Bifidobacterium longum* IMV B-7165 from the collection of industrial strains of Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine has been used in the study. It was isolated from the healthy human infant's gastrointestinal tract. To obtain biomass, cultivation was performed on Blaurock medium for 20–24 hours at a temperature of 37 ± 1 °C.

Clarification of the taxonomic position of the selected strain. Phylogenetic analysis of the selected strain 16S rRNA sequence was performed using BLAST software [17] and included detecting homologues and constructing a classical dendrogram of evolutionary relationships.

Sorbents. Commercial products "Enterosgel" (PJSC "ENVIRONMENTAL PROTECTION FIRM "CREOMA-PHARM", Ukraine), "Toxin.NET" (with inulin) (IlanPharm, Ukraine) and test item "Sillard P" produced by the OO Chuika Institute of Surface Chemistry of the National Academy of Sciences of Ukraine (trade name "Silix" OJSC "Biopharma") were used as highly dispersed silica sorbents.

Microscopy. To visualize *Bifidobacterium* microorganisms' sorption on "Toxin.NET", "Enterosgel", "Sillard P" sorbents microscopically bacterial culture and sorbents were mixed at room temperature until homogeneity. Samples were prepared for immersion microscopy with gentian violet staining, ×400. To obtain ×1000 microphotographs, the drop was dried on slide and examined in reflected light by microscope AXIO Observer A1M (Carl Zeiss company) with TopView 1000 camcorder.

Bifidobacteria immobilization on sorbents. The required amount of sorbent 1, 2, 3 and 5 g was added into flasks with 20 ml of bacterial suspension after cultivation (without centrifugation or after centrifugation) and stirred for 3 h at 37 °C for the most complete sorption on cells.

Freeze-drying of the complex probiotic product. To study the bifidobacteria stability after immobilization and freeze-drying, 2 ml samples were added to vials for freeze-drying. To protect the samples during freeze-drying special protective medium (sucrose 10%, sodium citrate 5%, skimmed milk powder 5%) was also added in ratio 1:2.

Drying was carried out on a freeze dryer TG15 in the following modes:

– temperature at the beginning of the process –(–60 ± 2) °C for 17 h;

– temperature at the end of the process +(30 ± 2) °C for 11 h;

– residual pressure ≤13.3.103 Pa;

– time of the process: 28 h.

After drying, samples dilution was performed into tubes with Blaurock medium.

Viability control. Viability control was carried out by Koch dish method in 3 days – via colonies counting after applying the appropriate dilutions on Petri dishes with hydrolyzed agar and cultivating at a temperature of 37 °C.

Modeling the upper gastrointestinal tract conditions **[18].** The bifidobacterial preparations with 5% and 10% sorbent concentrations were exposed to hydrochloric acid at pH 2 and pH 3, which corresponds to the human gastric juice acidity. For a clear confirmation of the pH value, it was adjusted to 2.0 and 3.0 by adding different volumes of 1 moles/l HCl and 5 mols/l HCl solutions. After 3 hours at 37 °C application on Blaurock solid medium was carried out. The colonies were counted on the third day.

To reproduce the conditions of the duodenum bifidobacterial complex products with 5% and 10% sorbent concentrations and control samples of bifidobacteria culture were applied on Blaurock medium with 20%, 40% and 60% of medical bile and kept at 37 °C for 3 h. The colonies were counted on the third day.

Statistical methods. All analyzes were repeated 3 times and performed in duplicate. The obtained data were expressed as the mean ± standard deviation and analyzed by Microsoft Excel 2010 software (Microsoft, Redmond, USA). Differences were considered to be statistically significant at $p < 0.05$.

The authors followed bioethical guidelines and recommendations of the International Committee of Medical Journals Editors (www.icmje.org).

Results

Taxonomic position determination of the Bifidobacterium longum strain selected for immobilization. Bifidobacterial isolate "4202", deposited as *Bifidobacterium longum* IMV B-7165, 16S rRNA sequencing and decoding were carried out.

Using BLAST, with a universal primer 5-AGAGTTTGATCCTGGCTCAG-3 (8-27) for 16S rRNA, phylogenetic analysis of this isolate section was performed. Results of sequence alignment of bifidobacterial isolate 16S rRNA allowed to suppose that the tested isolate was phylogenetically closer to *Bifidobacterium animalis subsp lactis* than to *Bifidobacterium longum*, to which it was previously attributed due to its physiological, biochemical and cultural characteristics, which might be due to the methodological possibilities available at the moment.

Indeed, the highest sequence "4202" alignment score, used for the studies, calculated for 4 strains of *Bifidobacterium animalis subsp lactis* was 968 (Fig. 1a), while for *Bifidobacterium longum* – 893 (Fig. 1b).

The evolutionary distance calculation confirms this conclusion, because for *Bifidobacterium longum* it is an order of magnitude greater than for *Bifidobacterium animalis subsp lactis* (Fig. 2).

Microscopic studies of silica samples with probiotic strains. Samples of *Bifidobacterium* with "Enterosgel", "Sillard P" and "Toxin.NET" were examined microscopically by different methods (with immersion and in reflected light) (Figs. 3, 4). The resulting images showed that the bacterial cells were immobilized on different silica samples. It should be noted that the character of previously studied *Streptococcus thermophilus* culture immobilization on "Enterosgel" and "Sillard P" (Fig. 6* [14]), did not fundamentally differ from immobilization of *Bifidobacterium*. Cells mainly were clustered around sorbents particles, which could be the evidence of their possible direct interactions.

Determination of sorbed bifidobacteria viability. A key step in the development of complex products based on immobilized cells is a choice of an effective carrier, which defines the effectiveness of microorganisms' protection from the negative effects of aggressive liquids. In order to study the effect of immobilization on the preservation and properties of bifidobacteria, were used the following carriers: "Enterosgel", "Toxin.NET" and "Sillard P".

For this aim, a suspension of the culture fluid with hydrogels was kept for 3 hours at a temperature of 37 °C (Fig. 5) with periodic stirring, which partially mimicked the research of other authors on the introduction of sorbents in the culture medium during cultivation.

Immobilized bifidobacteria vitality rate at gastrointestinal tract conditions modeling. The main aim of probiotics immobilization is their cells protection during the upper gastrointestinal tract passage. The hydrochloric acid action is extremely destructive for probiotics, which is confirmed by the control samples (without immobilization) vitality rates (Fig. 6). Our results show that immobilized cells

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bifidobacterium animalis subsp. lactis strain 952 16S ribosomal RNA gene, partial sequence	Bifidobacterium animalis subs...	968	968	100%	0.0	99.45%	1435	MT585610.1
Bifidobacterium animalis subsp. lactis strain 887 16S ribosomal RNA gene, partial sequence	Bifidobacterium animalis subs...	968	968	100%	0.0	99.45%	1429	MT585567.1
Bifidobacterium animalis subsp. lactis strain 1191 16S ribosomal RNA gene, partial sequence	Bifidobacterium animalis subs...	968	968	100%	0.0	99.45%	1434	MT573654.1
Bifidobacterium animalis strain ban1-6101-FNS-D20-50 16S ribosomal RNA gene, partial sequence	Bifidobacterium animalis	968	968	100%	0.0	99.45%	1441	MT902940.1
Bifidobacterium animalis subsp. lactis strain 3224 16S ribosomal RNA gene, partial sequence	Bifidobacterium animalis subs...	962	962	100%	0.0	99.27%	1438	MT613561.1
Bifidobacterium animalis subsp. lactis strain 2601 16S ribosomal RNA gene, partial sequence	Bifidobacterium animalis subs...	962	962	100%	0.0	99.27%	1445	MT611625.1

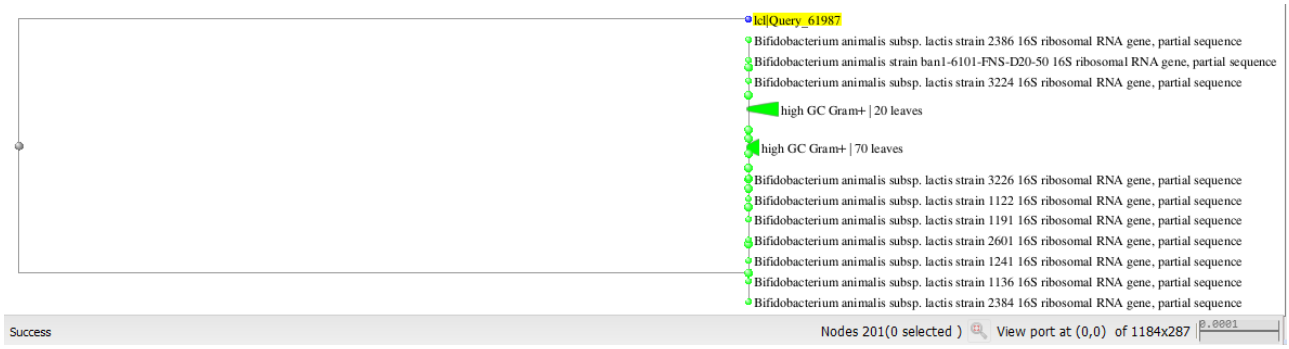
a

select all *100 sequences selected* GenBank Graphics Distance tree of results New MSA Viewer

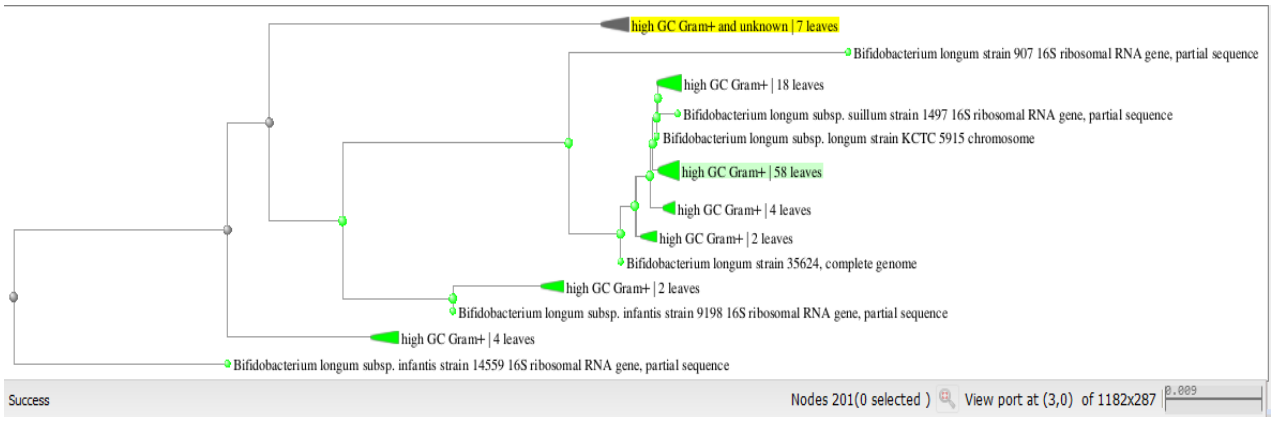
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bifidobacterium sp. 16S and 23S rRNA genes and ITS	Bifidobacterium l...	951	951	99%	0.0	98.90%	2013	X89111.1
Bifidobacterium infantis 16S rRNA gene, strain Y1	Bifidobacterium l...	928	928	99%	0.0	98.18%	1472	AJ311604.1
Bifidobacterium longum subsp. longum strain xswl666 16S ribosomal RNA gene, partial sequence	Bifidobacterium l...	893	893	91%	0.0	99.60%	1369	MZ675761.1
Bifidobacterium longum subsp. infantis strain xdm666 16S ribosomal RNA gene, partial sequence	Bifidobacterium l...	890	890	91%	0.0	99.60%	1365	MZ675762.1
Bifidobacterium longum subsp. infantis strain DSM 107246 16S ribosomal RNA gene, partial sequence	Bifidobacterium l...	859	859	99%	0.0	95.25%	1490	MN537525.1
Bifidobacterium longum 16S rRNA gene, strain Y10	Bifidobacterium l...	718	718	99%	0.0	89.58%	1479	AJ311606.1
Bifidobacterium longum subsp. infantis strain 2756 16S ribosomal RNA gene, partial sequence	Bifidobacterium l...	675	675	100%	0.0	87.59%	1429	M...

b

Figure 1: The results of the best alignments for the sequence of isolate "4202" 16S rRNA: (a) with the entire base of nucleotide sequences; (b) with *Bifidobacterium longum*

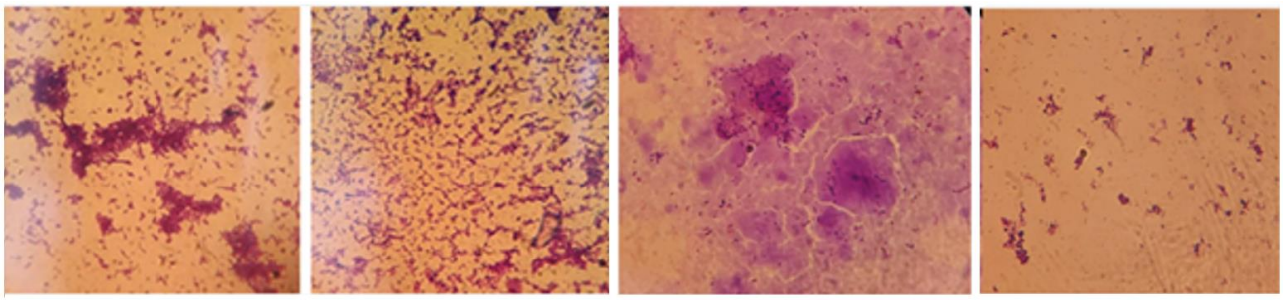


a



b

Figure 2: Classic dendrograms of evolutionary relationships based on the results of alignment for the sequences of bifidobacteria isolate "4202" 16S rRNA: (a) with the entire base of nucleotide sequences; (b) with *Bifidobacterium longum*



Enterosgel* Sillard P* Toxin.NET Sillard P

Figure 3: Microscopic image of streptococci (**Streptococcus thermophilus* – previous studies [14]) and bifidobacteria cultures with sorbents (×400)

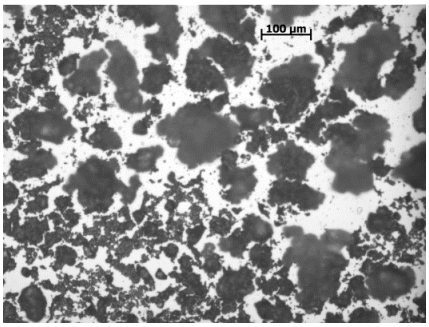


Figure 4: Microscopic image of *Bifidobacterium* cells sorbed on "Enterosgel" (reflected light, ×1000)

are better protected from acid. But it could be seen that with increasing acidity, both in control and immobilized cells vitality rates decrease. Thus, at pH 2 the number of bifidobacteria (immobilized with 5% and 10% sorbent) is 11 times lower than at pH 3. It is also noticeable that the difference in vitality rates for 5% and 10% sorbent – does not exceed the method error.

After passing the stomach with an extreme acidic environment, probiotic cultures together with food or in the form of a freeze-dried product enter into the duodenum, where are the bile ducts from the liver. Bile can also significantly destroy the microorganisms' cells, so the investigation of immobilized and intact bifidobacteria vitality rates in the presence of bile is of special interest. As can be seen from the obtained results test samples incubation for 3 h in medium with different concentrations of bile, their vitality rates are significantly reducing in control samples (Fig. 7). In the case of sorbent-immobilized samples of bifidobacteria, viability was higher. It must be noted that at all studied concentrations (20%, 40%, 60%) vitality rates of bifidobacteria, which contained 10% of the "Enterosgel", was significantly higher than in the control.

The vitality rate of immobilized culture with freeze-drying (Fig. 8) tended to decrease with

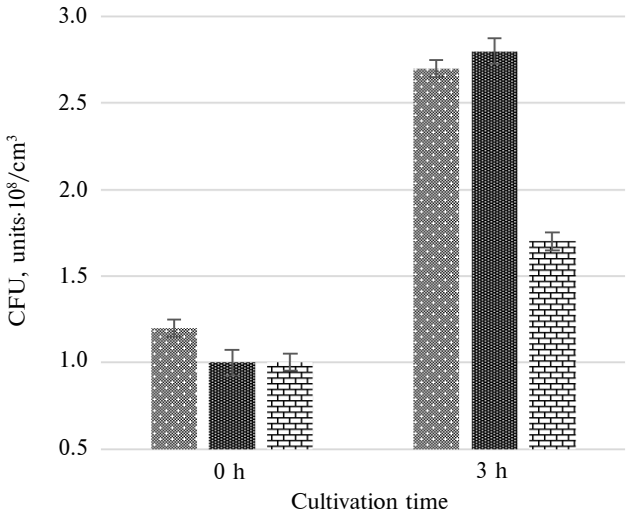


Figure 5: Diagram of the "Enterosgel"-immobilized bifidobacteria vitality rate: ▩ – control samples without sorbent, ▩ – samples with a 5% sorbent content, ▩ – samples with a 10% sorbent content

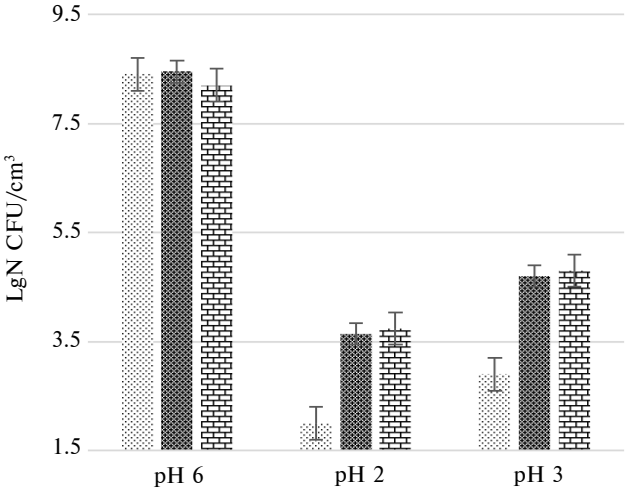


Figure 6: Vitality rates of "Enterosgel"-immobilized (5% and 10%) bifidobacteria probiotic strain during incubation at pH 6.0, pH 2.0 and pH 3.0, 3 h, 37 °C: ▩ – control samples without sorbent, ▩ – samples with a 5% sorbent content, ▩ – samples with a 10% sorbent content

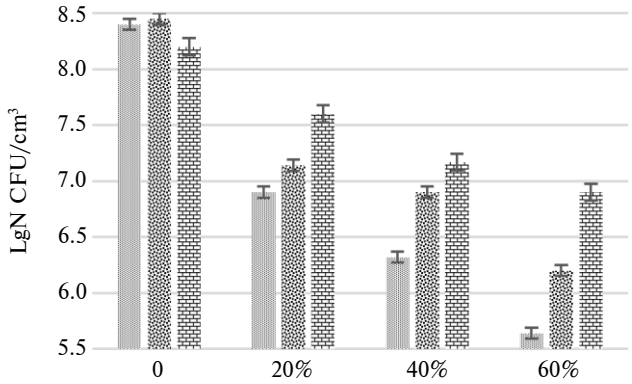


Figure 7: Vitality rates of "Enterosgel"-immobilized (5% and 10%) bifidobacteria probiotic strain during incubation at bile concentrations of 20%, 40% and 60%, 3 h, 37 °C: ▩ – control samples without sorbent, ▩ – samples with a 5% sorbent content, ▩ – samples with a 10% sorbent content

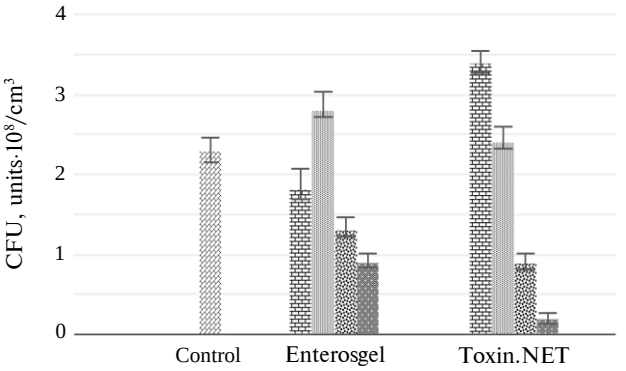


Figure 8: Vitality rates of bifidobacteria after freeze-drying in the case of adding from 5% to 25% of drugs "Enterosgel" and Toxin.NET: ▩ – samples with a 5% sorbent content; ▩ – samples with a 10% sorbent content; ▩ – samples with a 15% sorbent content; ▩ – samples with a 20% sorbent content

sorbent content increasing up to 25%. Such results confirm our previous data on the better survival of other lactic acid bacteria in combination with hydro gel ("Enterosgel") and not with xerogel ("Sillard P") [14]. On the whole the best results among the tested sorbents for bifidobacteria were observed in the case of culture immobilization with 5% of "Toxin.NET" – $3.4 \pm 0.7 \times 10^8$ CFU in comparison with control – $2.3 \pm 0.7 \times 10^8$ CFU. This effect could be realized due to protective action of inulin, which is a part of this preparation.

Discussion

In order to protect probiotic microbiota from bactericidal effects in the upper gastrointestinal tract we consistently used the following approaches: resistant strains of bifidobacteria selection (to provide ability to survive in acidic stomach juice and in aggressive mediums of the small intestine) and further their immobilization on different carriers' samples study.

In the development of biological products immobilized forms, the most important point is the choice of sorbent-carrier. Modern enterosorbents should be non-toxic, non-traumatic to the gastrointestinal mucosa, with high sorption capacity. Such properties have preparations of highly dispersed silica "Sillard P" ("Silix"), "Enterosgel" and "Toxin.NET", which are approved for enteral use.

Bifidobacteria introduction into the intestine contributes to its contents quantitative and qualitative normalization and stimulates the reparative process of the mucous membrane. Frequent dysbioses in children with dietary disorders caused by infections or antibiotics administration determined the choice of bifidobacteria (isolated from the gastrointestinal tract of the healthy human infants) as an object of study. It was confirmed that the provided 16S rRNA sequence of such an isolate belongs taxonomically to the genus *Bifidobacterium*, which was characterized by a high content of GC-nucleotides and gram-positive cell wall structure. Our bioinformatics analysis of this nucleotide sequence showed the greatest phylogenetic affinity of tested isolate to *Bifidobacterium animalis subsp lactis*, and not to the species *Bifidobacterium longum*, under which name this strain was deposited at the DK Zabolotny Institute of Microbiology and Virology of NASU.

As can be seen from the results of the immobilization conditions determination (see Fig. 5), the chosen method of immobilization of the culture suspension (3 h at 37 °C with stirring) for 5% and 10% sorbent content do not significantly reduce vitality rates: the number of colony-forming units remains at the same level as at the control before immobilization (10^8). This may be due to the absence of post-cultivation stress: cells remain in the culture medium at the cultivation temperature and are not washed after ultracentrifugation. The vitality rates reduction by 10% of the sorbent content is not significant given the order of numbers.

Numerous studies have focused on the resistance of immobilized probiotic microorganisms to the action of artificial environments modeling the human gastrointestinal tract conditions, as well as bacterial viability duration in food [19–21]. It was shown that immobilization of probiotic microorganisms allows to increase significantly the resistance of tested cultures to the action of these media. Immobilized preparations are superior in survival to traditional probiotics (liquid or dry cultures). It is also assumed that therapeutic and prophylactic effects of immobilized healthcare products are realized due to the combined actions of living bacteria, as well as protective and detoxifying properties of the enterosorbent itself. Immobilized bacteria are more resistant to environmental inactivating factors, including the gastrointestinal tract mediums [22].

According to many developers [23–26], sorbed products actively colonize the intestinal mucosa by creating a high local concentration of bifidobacteria, which allows them to better survive during the passage of the gastrointestinal tract and colonize its mucosa.

Our series of experiments to study the immobilized bifidobacteria acid resistance showed that the immobilization of cells on sorbents had a protective effect in conditions of low pH, and our results coincide with the results of other scientists [27].

To achieve the best result on the survival of immobilized bifidobacteria with gastrointestinal conditions modeling (pH 2, pH 3 and bile action), it seems appropriate to rationalize cell immobilization conditions and to modify the enterosorbent surface with a view to creating conditions both for sorption and protection of cells.

Regarding the decrease in the freeze-dried complex probiotic products vitality with increasing sorbent concentration, this effect is characteristic for the nanomaterial influences on living cells. It is possible that increase of surface tension contributes the destruction of the cell wall during drying. However, in our opinion, there may also be a purely methodological explanation for this fact. Cells seeded on the nutrient medium are surrounded

by sorbent particles and therefore have no physical possibility to contact to solid nutrient medium. This assumption is partly confirmed by studies of milk fermentation by a complex probiotic product which quite well "copes with the task", despite certain smaller number of living cells [14]. In a liquid medium, cells have greater access to the nutrient medium, so the functionality of the culture immobilized on the hydro gel may be better than encapsulated.

Inulin (which is a part of "Toxin.NET") helps to maintain just the bifidoflora, so this complex probiotic product was the best for preserve cells viability after freeze-drying of the immobilized test strain. For an optimal choice it is necessary to choose both concentration, and temperature, to apply other technological approaches which can influence on the choice of sorbent. Giving recommendation, the cost and availability of this sorbent must be also taken into account. So, inulin can be added into medium during immobilization simultaneously with "Enterosgel" to avoid the use of expensive pharmacopoeial healthcare product ("Toxin.NET" or similar). Furthermore, in case of cultures composition must be chosen parameters and sorbents providing the best vitality rates for each of them.

The obtained results will be used in the production of new fermenting healthcare products of the Institute of Food Resources of NAAS of Ukraine for sour milk product.

Conclusions

The taxonomic position of a bifidobacteria isolate (from the healthy human infants) used in immobilization studies was determined. Using taxonomic affiliation of microorganisms' identification procedure with the BLAST software and the 16S rRNA sequence it was shown that test item has the greatest phylogenetic affinity for *Bifidobacterium animalis subsp lactis* rather than *Bifidobacterium longum*.

With the upper gastrointestinal tract conditions modeling (in the case of acidity and bile) the best vitality rate was demonstrated by bifidobacteria cultures immobilized on the sorbent "Enterosgel" with a content of 10% (by weight of the culture).

Vitality rates of bifidobacteria immobilized on highly dispersed silica after freeze-drying was slightly reduced in the case of "Enterosgel" and "Toxin.NET" with their content from 15% to 25%. The best results were observed in the case of immobilization with 5% of "Toxin.NET" (combination of "Enterosgel" with inulin).

Interest disclosure

The authors declare no conflicts of interest to disclose.

References

[1] Berg G, Rybakova D, Fischer D, Cernava T, Vergès MC, Charles T, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020 Jun 30;8(1):103. DOI: 10.1186/s40168-020-00875-0

[2] Kho ZY, Lal SK. The human gut microbiome – a potential controller of wellness and disease. Front Microbiol. 2018 Aug 14;9:1835. DOI: 10.3389/fmicb.2018.01835

[3] Monsour HP Jr, Quigley EM. The microbiome: what will the future hold. Semin Liver Dis. 2016 Sep;36(4):354-9. DOI: 10.1055/s-0036-1594009.

[4] Starovoitova SO. Modern aspects of technology of immobilized probiotics. Biotechnologia Acta. 2012;5(4):9-20.

[5] Iravani S, Korbekandi H, Mirmohammadi SV. Technology and potential applications of probiotic encapsulation in fermented milk products. J Food Sci Technol. 2015 Aug;52(8):4679-96. DOI: 10.1007/s13197-014-1516-2

[6] de Paiva e Silva KK, de Souza Queirós M, Ribeiro APB, Gigante ML. Modified milk fat as encapsulating material for the probiotic microorganism Lactobacillus acidophilus LA3. Int Dairy J. 2022;125:105237. DOI: 10.1016/j.idairyj.2021.105237

[7] Babynets O.M. Properties of probiotics immobilized on enterosorbents after low-temperature storage. Bull Probl Biol Med. 2012;4(1):72-8.

[8] Haffner FB, Diab R, Pasc A. Encapsulation of probiotics: insights into academic and industrial approaches. AIMS Mater Sci. 2016;3(1):114-36. DOI: 10.3934/matersci.2016.1.114

[9] Kozlovska HV, Danylenko SG, Ibatullina FZ. Selection of protective media in the process of lyophilization of strains of bifidobacteria and lactobacilli. Sci J Nat Univ Life Environ Sci Ukr. 2012; 72(4):30-3.

[10] Gorbyk PP, Pentyuk OO, Shtatko OI. Prospects for the creation of combined drugs based on highly dispersed silica. Nanosyst Nanomater Nanotechnol. 2008;6(1):315-330.

[11] Kim SE, Zhang L, Ma K, Riegman M, Chen F, Ingold I, et al. Ultrasmall nanoparticles induce ferroptosis in nutrient-deprived cancer cells and suppress tumour growth. Nat Nanotechnol. 2016;11(11):977-85. DOI: 10.1038/nnano.2016.164

[12] Selvarajan V, Obuobi S, Ee PLR. Silica nanoparticles—a versatile tool for the treatment of bacterial infections. Front Chem. 2020 Jul 15;8:602. DOI: 10.3389/fchem.2020.00602
[13] Zaytsev V. Complexing silicas. Synthesis, graft structure and surface chemistry. Folio: Kharkiv; 1997. 233 p.
[14] Danylenko S, Romanchuk I, Marynchenko L, Kryzhska T, Nizhelska O, Potemska O, et al. Immobilization of probiotic cultures with enterosorbents based on highly dispersed silica. J Microbiol Biotechnol Food Sci. 2021;11(2):1-4. DOI: 10.15414/jmbfs.3334
[15] Danylenko S. Properties of microorganisms strains L. paracasei ssp. paracasei AND B. longum subsp. suis. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies Ser Veterinary Sciences. 2013;15(1):296-301.
[16] Knysh OV, Isaienko OI, Babych YM, Kompaniets AM, Pakhomov OV, Polyanska VP, et al. Antimicrobial activity of bifidobacteria derivatives after storage in a frozen state. Probl Cryobiol Cryomed. 2015;28(3):267-86. DOI: 10.15407/cryo28.03.237
[17] BLAST: Basic Local Alignment Search Tool [Internet]. Blast.ncbi.nlm.nih.gov. 2022 [cited 2021 Nov 23]. Available from: https://blast.ncbi.nlm.nih.gov/
[18] Boke H, Aslim B, Alp G. The role of resistance to bile salts and acid tolerance of exopolysaccharides (EPSS) produced by yogurt starter bacteria. Arch Biol Sci. 2010;62(2):323-8. DOI: 10.2298/abs1002323b
[19] Guergoletto KB, Magnani M, Martin JS, Andrade CGTdJ, Garcia S. Survival of Lactobacillus casei (LC-1) adhered to prebiotic vegetal fibers. Innov Food Sci Emerg Technol. 2010;11(2):415-21. DOI: 10.1016/j.ifset.2009.11.003
[20] Feklysova LV, Meskyna EO. A new generation of sorbed bifid probiotics in pediatric practice. Almanac Clin Med. 2005;8(1):329-38.
[21] Ding WK, Shah NP. Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. J Food Sci. 2007;72(9):446-50. DOI: 10.1111/j.1750-3841.2007.00565.x
[22] Ding WK, Shah NP. An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. J Food Sci. 2009;74(2):53-60. DOI: 10.1111/j.1750-3841.2008.01030.x
[23] Ding WK, Shah NP. Effect of various encapsulating materials on the stability of probiotic bacteria. J Food Sci. 2009;74(2):100-7. DOI: 10.1111/j.1750-3841.2009.01067.x
[24] Jayalalitha V, Balasundaram B, Palanidorai R. In vitro assessment of microencapsulated probiotic beads. Int J Agricult Res Rev. 2012;2(1):1-6.

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ВИКОРИСТАННЯ ВИСОКОДИСПЕРСНОГО КРЕМНЕЗЕМУ В БІОТЕХНОЛОГІЇ КОМПЛЕКСНОГО ПРОБІОТИЧНОГО ПРОДУКТУ НА ОСНОВІ БІФІДОБАКТЕРІЙ

Проблематика. Технологія іммобілізації пробіотиків є найбільш ефективним способом для контрольованої та безперервної доставки життєздатних клітин у кишківник. Як відомо, фізіологічна роль біфідобактерій багатопланова і полягає в нормалізації та стабілізації мікробіоценозу, формуванні колонізаційної резистентності кишківника, синтезі амінокислот, білків і вітамінів, підтримці неспецифічної резистентності організму тощо. Саме такий широкий спектр позитивного впливу на макроорганізм дає змогу розглядати біфідобактерії як основу для створення функціональних іммобілізованих здорових продуктів на їх основі.
Мета роботи. Визначення таксономічного положення вибраного для іммобілізації штаму біфідобактерій *Bifidobacterium longum*, дослідження виживаності біфідобактерій цього штаму в комплексному пробіотичному продукті на основі високодисперсного кремнезему за модельованих умов шлунково-кишкового тракту (ШКТ) та після ліофільного висушування.
Методика реалізації. Використовували виробничий штам *Bifidobacterium longum* ІМВ В-7165 із колекції промислових штамів Інституту продовольчих ресурсів Національної академії аграрних наук України. Штам виділено із ШКТ здорових немовлят. Використовували загальновживані біоінформатичні, мікробіологічні, біотехнологічні та статистичні методи.
Результати. Виконано й показано найкращі вирівнювання для послідовності 16S рРНК ізоляту біфідобактерій “4202”, (задепонованого як *Bifidobacterium longum* ІМВ В-7165) та класичні дендрограми на основі цих результатів. За результатами мікроскопічних досліджень зразків мікроорганізмів із високодисперсними кремнеземовими продуктами (“Ентеросгель”, “Силлард П” і “Токсин.NET”) встановлено, що іммобілізація культури *Streptococcus thermophilus* принципово не відрізняється від іммобілізації біфідобактерій. З метою вивчення впливу іммобілізації на збереження та властивості біфідобактерій було використано такі ентеросорбенти, як “Ентеросгель”, “Токсин.NET” і “Силлард П”. Виживаність іммобілізованих біфідобактерій додатково вивчали за модельованих умов ШКТ: іммобілізовані клітини краще захищені від дії кислоти та жовчі, хоча з підвищенням кислотності виживаність падає як у контрольних, так і в іммобілізованих клітин.
Висновки. Уточнено таксономічне положення ізоляту біфідобактерій, виділеного з організму здорових немовлят, який використовували в дослідженнях з іммобілізації (*Bifidobacterium animalis subsp lactis*). За модельованих умов верхніх відділів ШКТ у разі дії кислоти та жовчі найкращу виживаність продемонстрували іммобілізовані культури біфідобактерій на сорбенті “Ентеросгель” із вмістом 10 % від маси культури. Виживаність іммобілізованих препаратів після ліофільного висушування незначно знижувалась у разі іммобілізації на зразках ентеросорбентів “Ентеросгель” і “Токсин.NET” за їх вмісту від 15 до 25 % від маси культури. Найкращі результати спостерігали у разі іммобілізації біфідобактерій з 5 %-вим вмістом ентеросорбенту “Токсин.NET” (ентеросгель з інуліном).

Ключові слова: біфідобактерії; високодисперсний кремнезем; таксономічне положення; іммобілізація пробіотиків; здорові продукти.