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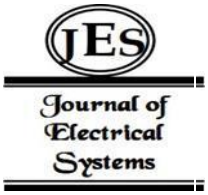
New Nutritional Supplement: Biotechnological Consortium of Microorganisms to Ensure the Gut Normal Microflora and Healthy Immunity



Abstract: - This research examines newly developed specialized goods. The composition of this biologically active nutritional supplement represents a consortium of live lacto-, bifido-, and propionic acid microorganisms: Bifidobacterium bifidum, Bifidobacterium animalis, Lactobacillus casei, Lactobacillus plantaruphm, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus, and Propionibacterium freudenreichii. Bacterial characteristics analysis helped determine the qualitative and quantitative composition of the product as well as its functional properties aimed at maintaining microflora and a healthy intestinal immune system. The manufacturing technology is characterized by microencapsulation, sparing parameters of a short and low-temperature drying process, microcapsules protecting the shell, and a prebiotic matrix of lactulose. All these factors make it possible to obtain biomass with a long shelf life to ensure its maximum survival, safety, and targeted implementation of microorganisms' functions. The regulated organoleptic and physicchemical indicators of product quality and safety have been determined. The content of bifidobacteria and lactobacilli must be at least 1·109 CFU/g. The product's shelf life has been determined following a thorough analysis of its microbiological, toxicological, and hygienic safety criteria. The recommended shelf life is one year, with a possible three-months extension at the temperature of 4±2°C and an air humidity of at least 60%. The product's functional properties, which help improve biocenosis and support healthy intestinal immunity, have been confirmed by the expert report of Russia's Federal Service for Supervision of Consumer Protection and Welfare. One capsule taken twice a day with a meal is advised for adults.

Keywords: Biotechnological product, Microbial consortium, Composition, Manufacturing technology, Improving microflora and intestinal immunity

I. INTRODUCTION

In the course of evolution, people have had to coexist with the microbiome, i.e. the collection of microbial genes (more than two million), including the gut microbiota [1-5].

Each person's microbiome is as unique as their fingerprints. It is seeded and modulated through evolution by numerous factors of the external and internal environment, the nutritional factor being fundamental. However, we can consider a modern human diet very poor for the gut microflora. The gut of an average person in Europe and the USA consists of 1200 different bacteria, while the gut of an Indian in the Amazon basin, whose lifestyle and diet are similar to those of their ancestors, consists of 1600 bacteria (plus 33 percent) [6-11].

Another example of a healthy microflora is that of the Hadza people in Tanzania, who are isolated from the outside world and have kept the traditions going back millions of years. Their diet, lifestyle, and gut microbiome have not changed much. They daily consume 100 to 150 grams of dietary fiber, a substrate for microorganisms, while

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Europeans and Americans consume only 10 -15 grams. As a result, we can observe qualitative and quantitative differences in their gut microflora composition, which affects the viability of the body's ecosystem [12-15].

The negative impact of civilization (malnutrition, ecology, stress, etc.) results in microbial flora disturbance and weaker immunity, which may be followed by the development and progression of autoimmune, common digestive and infectious diseases. Numerous results of placebo-controlled studies confirm this fact [16-19].

So we must improve the gut biocenosis efficiently by optimizing our diet with the help of specialized products, including nutritional supplements with targeted functional properties.

II. MATERIALS AND METHODS

The materials of the study are lacto-, bifido- and propionic acid bacteria, as well as their total community, used in the laboratory and experimental samples of the nutritional supplement. The microbial consortium and its mechanism of affecting metabolic processes are thoroughly examined in foreign studies [20-23].

Common and specialized methods of the specialized product quality assessment have been used in the study. The content of lacto-, bifido- and propionic acid bacteria has been determined according to Federal Standards P 56139 and 10444, and Procedural Guidelines 4.2.999.

III. RESULTS AND DISCUSSION

A microencapsulated biocomplex, a nutritional supplement, has been developed. It includes a consortium of live lacto-, bifido- and propionic acid bacteria to support the gut microbial flora and healthy intestinal immunity.

The community of probiotics consists of the following microorganisms: Bifidobacterium bifidum, Bifidobacterium animalis, Lactobacillus casei, Lactobacillus plantaruphm, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus, Propionibactirium freudenreichii.

Let's explore the attributes of the product's constituents, which dictate both its qualitative and quantitative structure.

Bifidobacteria and lactobacilli provide the basis of the primary normal colon biocenosis of newborns and normal flora of adults. This particular group of lactic acid bacteria accounts for 99 per cent of the entire gut microbial population of infants and up to 95 per cent in adults.

The common use of Bifidobacterium and Lactobacillus in probiotic products is explained by the presence of these prokaryotic taxa in normal biocenosis and their key role in the functioning of a healthy person's microbiological system.

Bifidobacterium and Lactobacillus play an important part in colonization resistance, digestion, metabolism of carcinogens, xenobiotics, cholesterol, and other vital functions. There are evidence-based studies on the positive effects of bifido-and lactoflora when treating inflammatory and infectious diseases of the gastrointestinal tract. By improving the gut flora, lactic acid probiotics enhance immunity, reduce the manifestations of food allergies and symptoms of lactose intolerance, and have antimutagenic, hypocholesterolemic, and anticarcinogenic effects.

Indigenous lactoflora enhances the body's anti-infective protection as it:

- Produces lactic acid and other antimicrobial and antibiotic-like substances – diacetyl, lysozyme, short-chain fatty acids (SCFA), hydrogen peroxide and lactacins (bacteriocins);
- Competes for adhesion sites and nutritional ingredients;
- Boosts the immune system.

Propionic acid bacteria also possess probiotic properties, cannot be digested in the gastrointestinal tract, are resistant to bile acids, and having low stomach acidity (pH 2.0). They inhibit the activity of nitroreductase, p-glucuronidase, and azoreductase, the gut microbial flora enzymes, which are involved in the formation of carcinogens, tumor growth promoters, and various mutagens. Propionic acid bacteria provide antimutagenic protection due to thiol compound accumulation, which contributes to preserving not only their own genotype but also that of other organisms.

Propionic acid bacteria produce a number of metabolites, which prevent the harmful impact of free radicals involved in the formation of mutagens and carcinogens, and binding their activated forms to DNA.

The paper demonstrates the antiviral and immunomodulatory properties of the probiotics under study. They are explained by the activation of natural killer cells, monocyte-macrophage system and interferon induction.

The dietary supplement under consideration also includes lactase enzymes to hydrolyze lactose to glucose and galactose, as well as papain to catalyze the breakdown of complex esters, proteins, amides, and peptides in food. These enzymes prevent the negative processes of fermentation and putrefaction in the gut, fungal microflora development, and allergies caused by toxins and undigested food as a result.

The manufacturing technology of the microencapsulated product has been developed. It is characterized by the following innovative features:

- Use of spray drying for microencapsulation allows obtaining the biomass with a long shelf life. Short and low-temperature drying ensures maximum survival of microorganisms;
- A microcapsule protecting shell ensures the probiotics acid resistance, and their ability to maintain viability and titer in the stomach acid environment (pH 1.5-2.0) for at least three hours.

The presence of microorganisms in the lactulose prebiotic matrix allows them to start dividing and functioning immediately after they get into the gut, regardless of its local conditions.

Figure 1 demonstrates probiotic bacteria, which are not microencapsulated, and **Figure 2** shows microencapsulated probiotic bacteria in lactulose matrix.

Enzymes in nutritional supplements are protected by an enteric coating. They are the first to be released from the shell, fermenting toxic metabolites and thus preventing their negative impact on probiotic bacteria survival and the gut colonization.

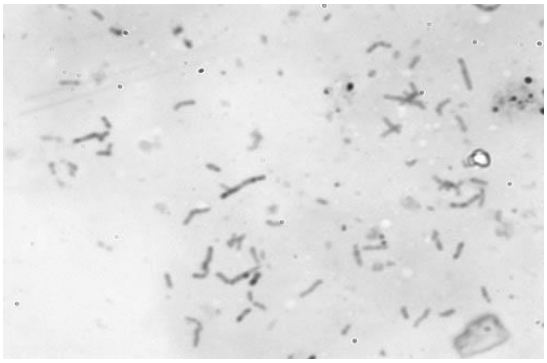

Figure 1. Not microencapsulated probiotic bacteria

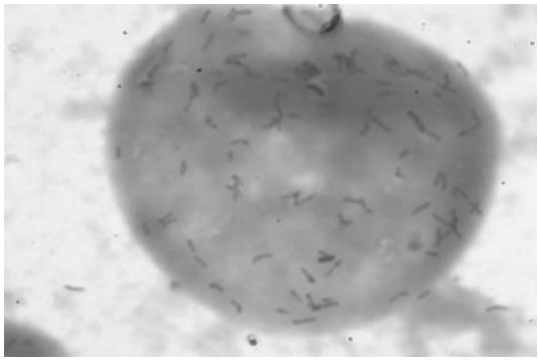

Figure 2. Microencapsulated probiotic bacteria in lactulose matrix

The other ingredients to ensure the probiotics viability in the product are inulin and Jerusalem artichoke, which provide fructooligosaccharides, cysteine, riboflavin, nicotinamide and para-aminobenzoic acid in the amounts

necessary for normal growth and development of the gut microflora. The product composition is presented in **Table 1.**

Table 1. Product composition.

№	Ingredient	Content, mg/1 capsule
1	Jerusalem artichoke tubers (powder)	100
2	Dry selective bacterial biomass BB-Bf of "Panbiom" series (Bifidobacterium bifidum)	80
3	Dry selective bacterial biomass BB-An of "Panbiom" series (Bifidobacterium animalis)	30
4	Dry selective bacterial biomass LB-Cs of "Panbiom" series (Lactobacillus casei)	60
5	Dry selective bacterial biomass LB-Pl of "Panbiom" series (Lactobacillus plantarum)	30
6	Dry selective bacterial biomass LB-Bg of "Panbiom" series (Lactobacillus delbrueckii subsp. bulgaricus)	30
7	Dry selective bacterial biomass LB-Ac of "Panbiom" series (Lactobacillus acidophilus)	20
8	Dry selective bacterial biomass PR-Frd of "Panbiom" series (Propionibactirium freudenreichii)	40
9	Lactase	20
10	Para- aminobenzoic acid	4
11	Papain	3
12	Cysteine	2.5
	Filler	
1	Inulin (carrier)	17.92
2	Neutral pellets (carrier)	30
3	Talc (anti-caking agent)	10
4	Silicon dioxide (Neosil GP) (anti-caking agent)	10
5	Calcium stearate (anti-caking agent)	5
6	Shellac (carrier)	4.8
7	Hydroxypropylmethylcellulose (carrier)	1.38
8	Nicotinamide	0.81*
9	Polyethylene oxide (carrier)	0.42
10	Riboflavin	0.07*
	Total content weight	500
	Hard gelatin capsule	
1	Gelatin (carrier)	99.85
2	Glycerin (moisturizing agent)	0.15

Total hard gelatin capsule weight	100
Total capsule weight	600

* included in the product composition in the amounts necessary for normal growth and development of the gut microflora

The manufacturing of the encapsulated nutritional supplement consists of the following stages:

- raw ingredients preparation. A 1 mm sieve is first used to filter the feedstock. It is subsequently smashed in a hammer mill and sieved once more;
- papain (semi-finished product) preparation. The process starts with layer-by-layer spraying of polyethylene glycol, hydroxypropyl methylcellulose, and papain on pellets in a fluidized bed. Before that, these ingredients dissolve in water when placed in a homogenizer reactor (RD-100). Then, the acid-resistant film coating is applied by an industrial granulator "Huttlin"TJ-200. Semi-finished granules are sieved through a 1 mm vibrosieve;
- preparation of the mixture for encapsulation. All the ingredients are measured and put together into a V-shaped mixer (C-300). After that, the mixture is made, smashed in a hammer mill, and then sieved one more time via a 1 mm vibrosieve. Look for lumps and inclusions in the mixture. At (4±2) ºC, the resultant mixture is stored for a maximum of 15 days;
- encapsulation is carried out by an automatic capsule filler, ZANASI 40E. The encapsulation process is controlled twice: first, every 30 minutes, 20 capsules are weighed to calculate the average weight and individual capsule weight with an error of ± 5%. Second, every 60 minutes, 10 capsules are examined for appearance. To meet the requirements a capsule must be smooth and even, without chips, dents, abrasion, and other faults. After evaluating the appearance, capsules are sent for packaging.

The manufacturing technology is considered innovative as it provides a microencapsulated nutritional supplement with a protective shell, which ensures the acid resistance of microorganisms in the stomach in an aggressive environment for minimum 3 hours. A short and low-temperature drying process ensures the maximum survival of bacteria. As microorganisms are contained in the prebiotic matrix, they start multiplying as soon as they get into the gut, even if there are no substances necessary for their growth.

After 15 months' storage period at the relative humidity of maximum 60% and temperature of 4±2 ºC, the product organoleptic and physiochemical studies were carried out to establish the regulated quality characteristics, including its nutritional value (**Table 2**). **Table 2** presents the regulated quality indicators of the product.

Table 2. Regulated quality indicators.

Indicator	**Characteristic**
Appearance	Gelatin capsule
Content color	White to beige powder with possible inclusions
Smell and taste	Specific
Capsule average weight, mg	600 (540-660)
Bifidobacteria content, CFU/g, minimum	$1 \cdot 10^9$
Lactobacilli content, CFU/g, minimum	$1 \cdot 10^9$

The product safety criteria after the expiration date have been studied. Microbiological, and toxic and hygienic criteria of the nutritional supplement safety have been examined according to the requirements of normative documentation (**Tables 3** and **4).**

Table 3. Microbiological criteria of the product safety.

Indicator	Value	
	Permissible value	**Actual value**
E.coli	1.0	Not detected
S.aureus	1.0	Not detected
Patagenes, including salmonella	10	Not detected
coliforms	0.1	Not detected
Yeast and molds, CFU/g, maximum	100	Maximum 10

Table 4. Toxicological and hygienic criteria of the product safety

Indicator		mg/kg, maximum	
		Permissible value	**Actual value**
Toxic elements	lead	0.1	Not detected
	arsenic	0.05	Not detected
	cadmium	0.03	Not detected
	mercury	0.005	Maximum 0.001
Pesticides	HCH	0.05	Maximum 0.005
	DDT and its metabolites	0.05	Maximum 0.005
	Heptachlor	Maximum 0.002	Maximum 0.002
	Aldrin	Maximum 0.002	Maximum 0.002

The presented results indicate the developed product hygienic safety after the expiration date. The shelf-life of one year with a possible 3 months extension is determined, if the product is stored under the specified conditions.

IV. CONCLUSION

The nutritional supplement is appropriate as a source of bifidobacteria, lactobacilli and propionic acid microorganisms to improve the gut biocinosis and ensure its healthy immunity. The expert assessment from Russia's Federal Service for Supervision of Consumer Protection and Welfare (Rospotrebnadzor) has validated these functioning attributes. Adults should take one capsule with a meal twice a day at the prescribed dosage.

The customized product's manufacturing procedures and composition have undergone testing at ArtLife Company's businesses and have been verified in compliance with integrated quality and safety management system criteria (ISO 2001, 22000, GMP standards).

Acknowledgments: The team of authors thanks the administration of the « Art life» company for the opportunity to research its basis.

Conflict of interest: None

Financial support: None

Ethics statement: The study was conducted according to the guidelines of the Declaration of Helsinki.

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