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COMPOSITION AND TECHNOLOGY OF POWDER WITH PROBIOTIC EFFECT MADE FROM LOCAL RAW MATERIALS

Namozov F.Sh., Yusupova G.R.

Tashkent pharmaceutical institute, Tashkent city, Republic of Uzbekistan

E-mail: farrukhjonnamozov@gmail.com

ABSTRACT

One of the main issues in the production of drugs with high bio-efficiency, which can replace imported drugs on the basis of raw materials obtained from microorganisms using modern methods, is the development and standardization of scientifically based quality control analysis methods. Due to the fact that medicines contain a large amount of different bioactive substances, the standardization of such medicines causes certain difficulties. The chemical composition obtained with the help of microorganisms has a generalized effect on the whole organism, not only occupying or filling the metabolic processes of its components, it does not change the speed of physiological flow, but also controls subtle pathological changes.

Key words: *Lactobacillus spp, probiotic powder, probiotics, assortment, Bifidobacteria, production, testing methods.*

Benefits: Bifidobacteria are the main component of healthy intestinal microflora, and they are mainly found in the large intestine. The most important function of bifidobacteria is protection. Microorganisms for this:

- production of substances that maintain proper acidity in the gastrointestinal tract and provide a bactericidal environment;
- specific inhibition (inhibition) - synthesis of molecules that block the growth of pathogenic pathogens;
- responsible for resistance to intestinal infections;
- stimulating the production of protective immunoglobulins;
- prevents the development of putrefactive microorganisms;
- reducing the production and release of histamine, thereby preventing the development of food allergies.

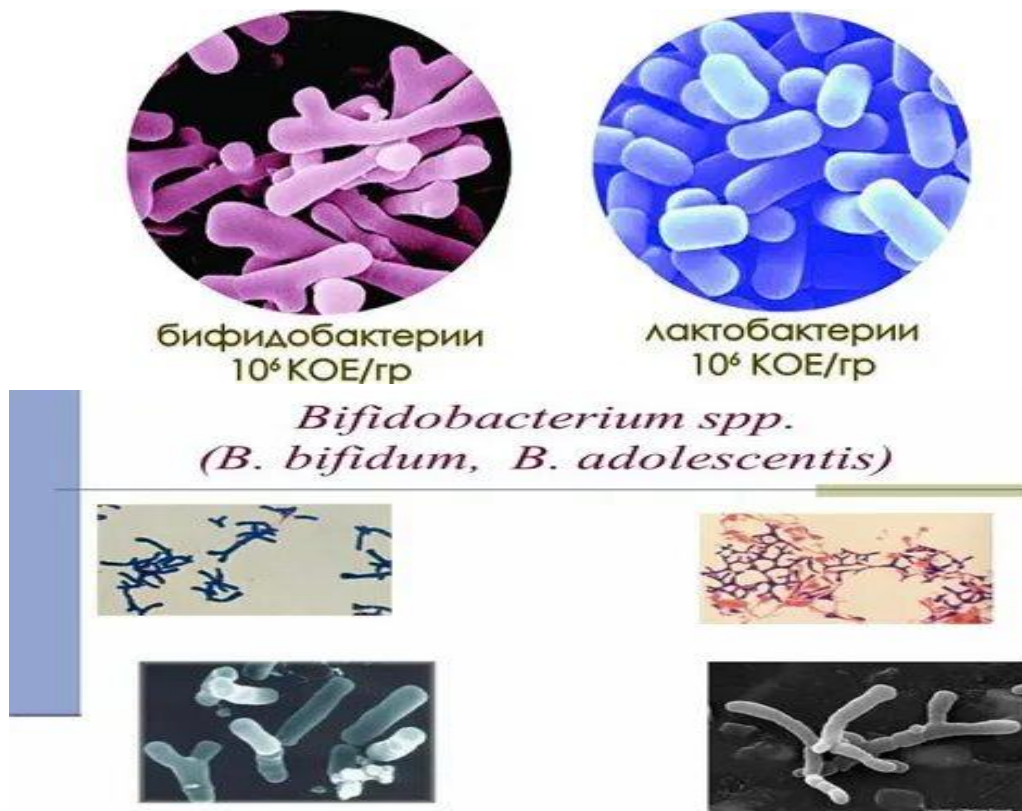
The second important function of bacteria is synthesis. They are necessary for the formation of vitamins in the intestines: B1 (thiamine), B2 (riboflavin), B6 (pyridoxine), nicotinic and folic acid, vitamin K. Beneficial microorganisms also synthesize the

necessary amino acids, including tryptophan, which is necessary for the formation of the "hormone of happiness" serotonin. Bifidobacteria help break down fats and absorb nutrients, improve intestinal motility and thus reduce the risk of constipation.

Purpose of the study: Lactobacilli are lactic acid bacteria that increase the acidity level of the environment and create unfavorable conditions for the development of pathogenic microorganisms. Their number is much smaller than bifidobacteria, but lactobacteria perform equally important functions:

- manifestation of growth of putrid and opportunistic microorganisms;
- release of natural antibacterial substances;
- activation of cellular immunity and production of immunoglobulins;
- participation in the breakdown of nutrients, including fats and lactose.

Fecal microflora analysis is performed to determine the number of different types of bacteria and their proportions. This helps to detect intestinal dysbiosis in time and to carry out the necessary treatment. Taking Bifidobacterium longum is useful for: Lactobacillus and bifidobacteria complex, in particular Bifidobacterium longum, is generally considered safe for most people, but it will not be superfluous to consult a doctor about taking it. Especially when it comes to their use in children under 12 years old, pregnant women and people with weak immunity, these tools cause a lot of problems.



Picture 1 Bifidubacterium spp. Microscopic structure

Method and methods: Extraction (Latin *extraho* - to extract, to extract) is a process of extracting important biologically active substances from plant and animal raw materials with the help of selected bacteria (microorganisms).

The choice of extractant is determined by the degree of viability and reproduction of the substance to be extracted. Medium for extracting substances from *Bifidobacterium longum*: water, sugar, glycerin; organic solvents are used for non-polar ones. Polysaccharides, moist and moderate heat-providing factors are also used. As a result of mixing *Bifidobacterium longum* with water, it is possible to prepare different polar solvents and selectively separate biologically active substances. The possibility of obtaining a large amount of Biologically Active Substances (BAS) complexes with the help of microorganisms is very high. But none of them can show the unique properties of the raw materials that give a special probiotic property, their effect is manifested only in general. In the same way, bifidobacteria are also active in preventing diseases.

The increasing need for the use of lactobacilli in industry and the expansion of the market for probiotics have led to the search for new economic fermentation media to obtain high yields of these bacteria. The goal of the next study was to develop a growth medium for *Lactobacillus*. Its main ingredients were wheat, corn, barley and rye flour. The optimum temperature, water content and pH level of the medium for the growth of lactobacilli in a new medium for semi-solid fermentation (SSF) were analyzed by tablet counting method. It was found that the highest number of bacteria was obtained when grown in semi-solid fermentation (SSF) medium with a flour to water ratio of 1:1.5 at a natural pH of 6.0 at 37C⁰. then the growth kinetics of the analyzed strains in semi-solid fermentation (SSF) medium was studied. The new medium helped increase the duration of the lag phase from 1.98 to 5.64 in selected lactobacilli strains. In addition to the composition of raw materials with probiotic effect, the growth of *Lactobacillus*, *Bifidobacterium longum* can be influenced by a number of different conditions, such as temperature, pH, oxygen concentration or water activity. The optimal temperature and pH conditions for the growth of lactobacilli are 30-40C⁰ and 5.5-6.2, respectively; however, the genus *Lactobacillus* is diverse and its bacteria can grow at 2 to 53 C⁰ and pH 4.5 to 6.5, and some strains can grow at even lower pH. Cultivation conditions, as well as the composition of the fermentation medium in which *Lactobacillus*, *Bifidobacterium longum* bacteria are grown, can affect the parameters of growth kinetics, for example, the specific growth rate and the duration of the lag phase, during which these bacteria adapt to a new environment and do not multiply time counts.

Results: The effect of individual, purified components isolated from *Bifidobacterium longum* is weak. The overall effect of biologically active substances

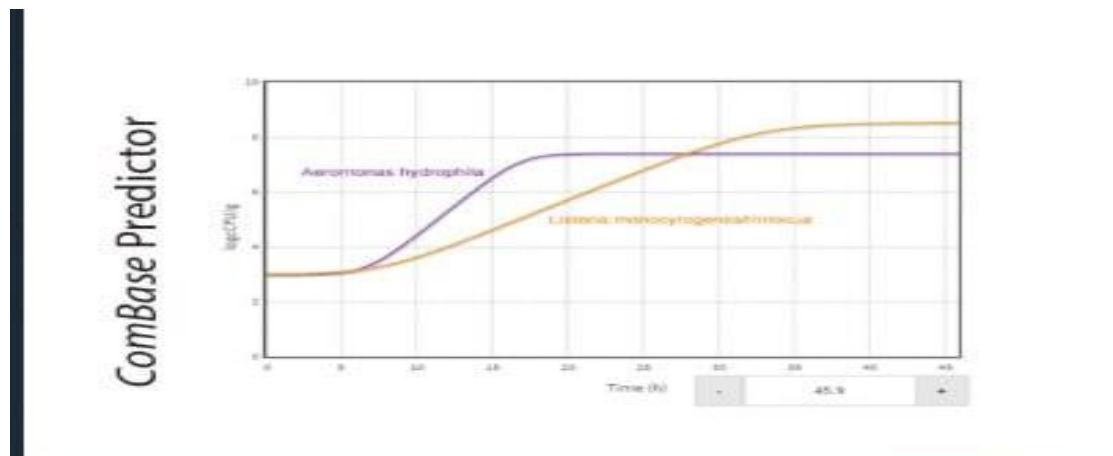
contained in the powder, capsule and dry extract of complex compounds used as probiotics is considered to be much more effective than the effect of individual components.

These technologies have several disadvantages:

- used probiotics cannot separate a large group of fat-soluble biologically active substances, water-soluble substances are not deeply extracted, that is, the complex action mechanism does not work 100%;
- methods of obtaining microorganisms in specially equipped laboratories are used as raw materials, which limits the capabilities of medium-level enterprises;
- during the drying process of raw materials with a probiotic effect, the quality of biologically active substances deteriorates under the influence of high temperature, and their loss during evaporation is observed.

Therefore, it is urgent to develop new complex technologies for obtaining products from the raw materials of microorganisms with a probiotic effect, and the selection of different separators during extraction is an important link of research.

Probiotic strains were cultured in Semi-Solid Fermentation medium and Mrs broth for comparison at temperature and pH for 30 hours, determined during inoculation and after 4, 8, 12, 16 hours, 24 and 30 hours of incubation. In addition, the pH of the culture medium was measured at the same time points. The assay was performed in triplicate. Growth curve evaluation was used to determine the maximum growth rate (μ_{max}) and duration of the lag phase (λ) and to calculate the production time (GT) for each strain grown in each medium.



Picture 2. A view representing the growth rate of microorganisms

Effects of temperature and pH on the growth of Lactobacillus. Strains were introduced into test tubes with Mrs broth or semi-solid fermentation (SSF) medium with a standardized pH value of 6.2 ± 0.2 and modified initial pH values of 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5. To determine the effect of temperature on the growth of strains,

tubes with inoculums of standard pH were incubated at 4, 20, 30, 37, 44, and 55°C for 24 hours. The effect of pH on the growth of the strains was determined after 24 hours of cultivation at 37°C.

After incubation, serial tenfold dilutions were prepared and cell numbers were determined by enumeration on MRS-agar tablets. 1 g of each sample from semi-solid fermentation (SSF) medium was suspended in sterile saline (10 ml) and homogenized before dilution. The plates were then incubated at 37°C for 48 h, and then the number of bacteria was counted as CFU/g or CFU/ml.

$$VCP = \frac{X}{t},$$

Picture 3 Formula for measuring the number of bacteria in the incubation process

Each experiment was performed in triplicate and the number of bacteria was recorded as CFU per gram (CFU/g). In addition, results were optimized with modifications by calculating viable cell productivity (VCP) (1) according to Ming et al.

Conclusions: One of the main issues in the production of drugs with high bioefficiency, which can replace imported drugs, based on raw materials with probiotic effect obtained with the help of microorganisms, is the development and standardization of scientifically based quality control analysis methods. Due to the fact that medicines contain a large amount of different bioactive substances, the standardization of such medicines causes certain difficulties. The chemical composition beneficial to the body with a probiotic effect obtained by microorganisms has a general effect on the whole body, not only occupying or filling the metabolic processes of its components, it does not change the speed of physiological flow, but also controls subtle pathological changes.

3 types of probiotic drug forms: powder, capsule and dry extract forms were studied. This drug form is obtained from raw materials with probiotic effect obtained by microorganisms in special conditions. Having fully studied the technology of obtaining raw materials with probiotic effect from microorganisms, their drug form and use, as well as the mechanisms of action, were studied.