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NEW PROBIOTIC CULTURE STRAINS IN THE PRODUCTION OF FERMENTED DAIRY PRODUCTS

Summary. In recent years, there has been a growing interest in products that benefit the body. Probiotics, containing live strains of the natural human microbiota, are one of the most important categories of functional foods today. First generation probiotics such as Bifidobacterium, Lactobacillus and Propionibacterium have been used for a long time. In addition, these bacteria are used in the production of various healthful dairy products. The functional foods industry is expanding as more and more people become ill and their immune systems decline. Due to the decrease in beneficial microflora, the number of pathogenic and moderately pathogenic microorganisms and their metabolites increases in the human body. As a result, the intestinal flora is altered, leading to allergies, cancer and anaemia. Special attention should therefore be paid to the microbial balance of the gastrointestinal tract, which is essential for human survival, by eating organic foods rich in probiotic microflora. Organic functional foods with probiotic microflora are a sensible way to maintain and improve human health. The resistance of strains of lactic acid bacteria was assessed 'In vitro' under conditions approximating the functioning of the human digestive system. We identified strains that can be used as probiotics in the production of healthy foods.

Key words: strains, probiotics, bacteria, probiotic culture, dairy products, enzymes, microorganisms.

Formulation of the problem. In recent years, the market for fermented foods has grown dramatically and lactic acid bacteria have become an integral part of their production technology. This affects the properties and quality of the final product. In recent years, there has been a growing interest in functional foods that affect the whole body or specific organs or systems. Probiotic products are distinguished from other functional food ingredients by the addition of live microorganisms (which act as antagonists against pathogenic or undesirable human gut microflora) that help manage gut microflora. Probiotics come in different forms. These are beneficial microorganisms (non-pathogenic and non-toxic) that can have beneficial effects on the human body when regularly introduced into the diet in the form of preparations or food components. There are many different strains of lactic acid bacteria: Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus Firmamentum, Lactobacillus rhamnosus. You can buy mono-probiotics - probiotics consisting of only one species of bacteria, or related probiotics consisting only of different species of bacteria (from 2 to 30 species). Probiotic bacteria synthesize amino acids and enzymes, help in overall metabolic functions, compensate for the lack of animal protein and promote digestion and absorption of food. Probiotics can contribute to the physiological activities and biochemical reactions of organisms in two ways: directly by changing the metabolic activity of cells in the organs and tissues they affect, and indirectly by modulating the biofilm function of microorganisms in the mucous membrane. A number of probiotics can benefit the human body by modifying the immune system, macrophage activity and restoring the microenvironment. In addition, they can promote colony-forming resistance by increasing colony-forming resistance and preventing the transmission of potentially harmful micro-organisms through the mucous membrane. Probiotics and functional foods containing live microorganisms benefit humans by normalising gut microbiota, modulating biochemical reactions and physiological cell activity, and regulating homeostatic processes of the immune, endocrine and nervous systems. This is why they are essential [11].

Today, a variety of probiotic dairy products are produced and used all the time. Probiotics benefit the body by promoting the growth and metabolism of beneficial bacteria in the stomach. Products that already contain probiotic bacteria can be supplemented with probiotics (e.g. yoghurt or baby food supplements). Probiotics promote the growth and movement of beneficial bacteria in the stomach, making the food more valuable to the body. Some of the ingredients include disaccharides and trisaccharides, oligosaccharides, polyols, amino acids, enzymes, low molecular weight fatty acids, antioxidants, beneficial plant extracts and microorganisms. Probiotics are not broken down by digestive juices or enzymes in the upper digestive tract, so they can enter the environment unhindered by bifidobacteria and lactic acid bacteria. Probiotics produce lactic acid that interacts with minerals, promoting their absorption in the stomach. Lactic acid also makes calcium and magnesium lactates more soluble and easier to digest. They also help regulate cholesterol and blood sugar levels, and bind and remove potentially harmful chemicals from the diet.

The human gut microbiota is a fragile component of the body's microbial ecosystem, and maintaining a healthy digestive tract is important for overall health. One of the most important challenges in this field is the development of new and effective probiotic products and probiotic formulations. Probiotic products are produced by biotechnological processes that start with the cultivation of bacteria belonging to different taxonomic groups. The microflora present in the raw material affects the direction of the biochemical reactions that occur in it. This has a significant impact on the quality and safety of the products used in production. In this case, it is all the more important to select strains that meet certain parameters, evaluate the duration of the manifestation of their properties and improve the biotechnology of biomass probiotic cultures used in food production [8].

Stable fermenting microorganisms are increasingly being used in the food business, including specialised fermented products made from a wide range of raw materials. More and more companies are using starters to produce high quality dairy products, and they can be used in a variety of ways. In the production of healthy fermented dairy products, it can be difficult to find a starter culture that is compatible with the materials and techniques used in the dairy sector. There are several problems with obtaining probiotic cultures for direct application. Synbiotic and probiotic technologies have facilitated the development of direct application starters, allowing them to be applied to more complex raw materials and to modify the inherent activity of the microorganisms and the products they produce. In addition, existing methods of producing probiotic cultures do not take into account all the physiological characteristics of the human digestive system. As a result, the microbial cells die and the ability of the bacteria to take root in the human intestine is reduced. A favourable combination of factors must be present in starter cultures and products derived from them for probiotics to work well in the human body [1].

Microorganisms can be cultured in different ways to biotechnologically produce strains with different characteristics. This makes it possible to develop one-of-a-kind products that take advantage of the properties of microorganisms.

A more efficient way of selecting strains is to look for strains that can retain their biochemical activity for a long time. If the culture does not contain enough biochemically active cells, it will not be as useful and healthy as it could be. On the other hand, those who create fermented products with lactobacilli rely heavily on the efficiency of the strain and the number of bacteria present in the final product [7].

There are several bioactive compounds produced by microbial probiotics. These, together with vitamins and enzymes, contribute to overall human health by aiding digestion and metabolism and providing essential nutrients to the body.

When selecting a functionally viable strain, it is important to consider the amount of energy required for acid production, the amount of salt in the diet, the availability of bile and the rate of change in the ph of the diet.

Despite this progress, the search for indigenous lactic acid bacteria with a competitive advantage for use in food continues and objective criteria for probiotic characterisation are being developed. Objective criteria for the evaluation of probiotic properties are also being established, providing a technical route for the development of functional foods for health maintenance [3].

Probiotic bacteria must comply with a number of rules in medicine. According to experts, microorganisms must generally be non-pathogenic and non-toxic, be able to pass through the gastrointestinal tract, adhere to and colonise intestinal epithelial cells, and have metabolic and therapeutic activity in the gut ecosystem. This refers to the emergence of "acidophilic" and "bile acidophilic" bacteria [1].

A number of regulations governing probiotic products and how food companies produce them are reflected in legislation. In the food business, bacteria are classified according to morphological, physiological, biochemical and technical criteria. Gram staining, temperature and pH optimisation for growth, growth in hydrolysed milk containing nitrogen and bile, acid-producing activity, carbon dioxide and ammonia synthesis from arginine, lactic acid fixation, carbohydrate fermentation, alcoholic fermentation, etc. are investigated.

Serological and phage typing procedures are used to confirm the presence of bacteria. Lactic acid bacterial cultures are identified by molecular genetic profiling.

In this context, it is worth noting that microbiological identification criteria in the food industry are outdated. Anyone can become a consumer of probiotics only if they know that the products they buy contain beneficial microorganisms.

To be considered useful, a product must contain at least 106 CFU/cm3 of live cells. In some Western European countries, probiotics must contain a minimum of 107 CFU/cm3 to be considered safe.

A number of studies have shown that most probiotic cells are destroyed during storage or lose their effectiveness once they enter the gastrointestinal tract. Consumption of fermented dairy products lowers pH levels in the body and increases gastric acidity [5].

Intestinal fermentation produces indole, scatole and phenol, all of which act as inhibitors of the growth and development of beneficial micro-organisms. Only phenol-resistant lactic acid bacteria can develop in the intestine. The small intestine contains a large number of bacteria, which can be destroyed by bile. Lipids and fatty acids belonging to cell membranes are very sensitive to bile ions. This leads to the destruction of some bacteria.

Therefore, the ability of probiotic bacteria to cope with bile acids affects their efficacy (up to 40% in the presence of bile salts) [6].

Those interested in selecting productive strains should look for strains resistant to bile acids, phenols and low pH.

The aim of the study was to discover and isolate new strains of probiotic lactic acid bacteria cultures, such as Lactobacillus. We then assessed whether they could withstand conditions similar to those in the human digestive system, in order to determine their efficacy in such scenarios.

Analysis of recent studies. Studies of strains are reflected in the works mainly of many foreign scientists: M. Lema, L. Williams, D.R. Rao, Holzapfel W., Charteris W.

The authors of the cited publications studied the following: strains of Lactobacillus c. lmb. casei; lmb. plantarum; Lbm acidophilum; and Lactobacillus fermentum regulate the growth of intestinal microflora, are not destroyed under the influence of gastric juice and, administered orally, enter the intestine intact.

Formation of the goals of the article (problem statement). The purpose of the article is to discover and isolate new strains of probiotic cultures of lactic acid bacteria, such as Lactobacillus. We then assessed whether they can withstand conditions similar to those found in the human digestive system in order to determine their effectiveness in such scenarios.

Main part. The subject of the study is Lactobacillus c. Lmb. Casei; Lmb. Plantarum; Lbm acidophilum; Lmb fermentum; they were isolated directly from fermented milk and contain natural bacteria. Control strains were obtained from the probiotic complex of bifido-, lactobacilli and Saccharomycetes Probiz. Identification of microbial species based on morphological, cultural, physical and biochemical characteristics. Basic criteria: Gram staining, optimum and critical growth temperature, normal pH, high temperature in the presence of sodium chloride, egg yolk and carbon dioxide, degradation of carbohydrates and alcohols [2].

After incubation in sterile skim milk, acidity was used to determine strain activity and titratable acidity. Acidity was measured according to GOST 3624-92. Potentiometers according to DSTU 8550: 2015 were used to assess active acidity (pH). The coagulation ability of the milk was evaluated by the time of clotting initiation.

Bile resistance was tested after 24 hours of incubation in liquid medium containing 0.5%, 20% or 40% bile; the number of solid bacteria present per milliliter of solution was determined using Cole medicata (medicated bile).

Salinity tolerance was assessed using liquid media containing 2% to 6% inorganic substances; the ability of bacteria to grow in the presence of NaCl was measured using liquid nutrient solutions containing 2% to 6% NaCl. Salt tolerance was evaluated by biomass growth (change in optical density) and D value after 24 hours of incubation at maximum temperature [3].

The resistance of lactic acid bacteria to phenol was evaluated by multiplying the biomass growth (change in optical density) by the D value after 24 hours incubation at the optimum temperature using a liquid nutrient solution containing 0.4% phenol.

This research led to the discovery of a number of lactic acid bacteria belonging to different groups. From these, naturally occurring lactic acid bacteria (strains) (D) and commercially available lactic acid bacteria (PR) with similar properties were selected for further investigation: Lactobacillus casei, Lactobacillus plantarum, Lactobacillus Lactobacillus fermentum.

The results show that the characteristics of the strains tested for biomass collection vary according to the type and species of lactic acid bacteria. Remarkably, all strains gained biomass rapidly in the first 10-12 hours.

All cultures studied fermented well with milk (Table 1), with Lmb. casei, Lmb. plantarum, Lbm. acidophilum (Pr) forming solid masses of equal concentration. They were subjected to fermentation to impart flavour. In addition to whey, Lmb. fermentum and Lmb. brevis (D) strains were detected in the masses [4].

Table 1

Ability of lactic acid bacteria to produce lactic acid

Strain	Fermentation activity hour	Acidity			Number of viable cells CFU/cm^3
		Active pH	Titratable	Limiting	
Lmb. casei 1 (Пр)	6	4,64	106	154	$2,2\cdot10^{10}$
Lmb. casei 2 (Пр)	6	4,63	108	158	$1,8\cdot10^{10}$
Lmb. casei 3 (Д)	7	4,70	108	154	$1,0\cdot10^{10}$
Lmb. casei 4 (Д)	6	4,68	110	158	$5,3\cdot10^{9}$
Lmb. casei 5 (Д)	4	4,59	120	170	$3,0\cdot10^{10}$
Lmb. casei 6 (Д)	6	4,71	110	156	$1,8\cdot10^{10}$
Lmb. fermentum 1 (Пр)	7	4,70	104	142	$5,4\cdot10^{9}$
Lmb. fermentum 2 (Пр)	8	4,76	106	148	$9,5\cdot10^{9}$
Lmb. fermentum 3 (Д)	6	4,75	100	138	$3,6\cdot10^{9}$
Lmb. fermentum 4 (Д)	7	4,77	112	154	$2,4\cdot10^{10}$
Lmb. plantarum 1 (Пр)	6	4,71	104	176	$7,7\cdot10^{9}$
Lmb. plantarum 2 (Д)	6	4,70	102	178	$1,5\cdot10^{9}$
Lbm. acidophilum (Пр)	4	4,62	119	210	$2,1\cdot10^{9}$

It is worth noting that these 'wild' lactic acid bacteria share many technical characteristics with strains used in cheese production. Some strains produce more acid than those used in cheese-making: the most promising are Lmb. casei 5 (E), Lmb. fermentum 4 (E), Lmb. plantarum 2 (E).

Other strains are suitable for producing a range of cultured milk products because they produce sour milk at 100-120°C after 24 hours, besides being necessary for a variety of cultured milk products.

On dairy products, each strain examined formed a different peptide ring of casein in Eikman's agar medium, indicating their ability to produce enzymes that hydrolyse milk proteins. This indicates that these strains are suitable for use in the food industry. They have also been found to produce extracellular and cell-associated proteases, which are essential for proper protein metabolism in organisms [10].

In this study, data were collected on the survival of lactic acid bacteria under pH stress (Fig. 1), combined with 20% and 40% bile, and the highest concentration of bacteria was found in the intestine [9].

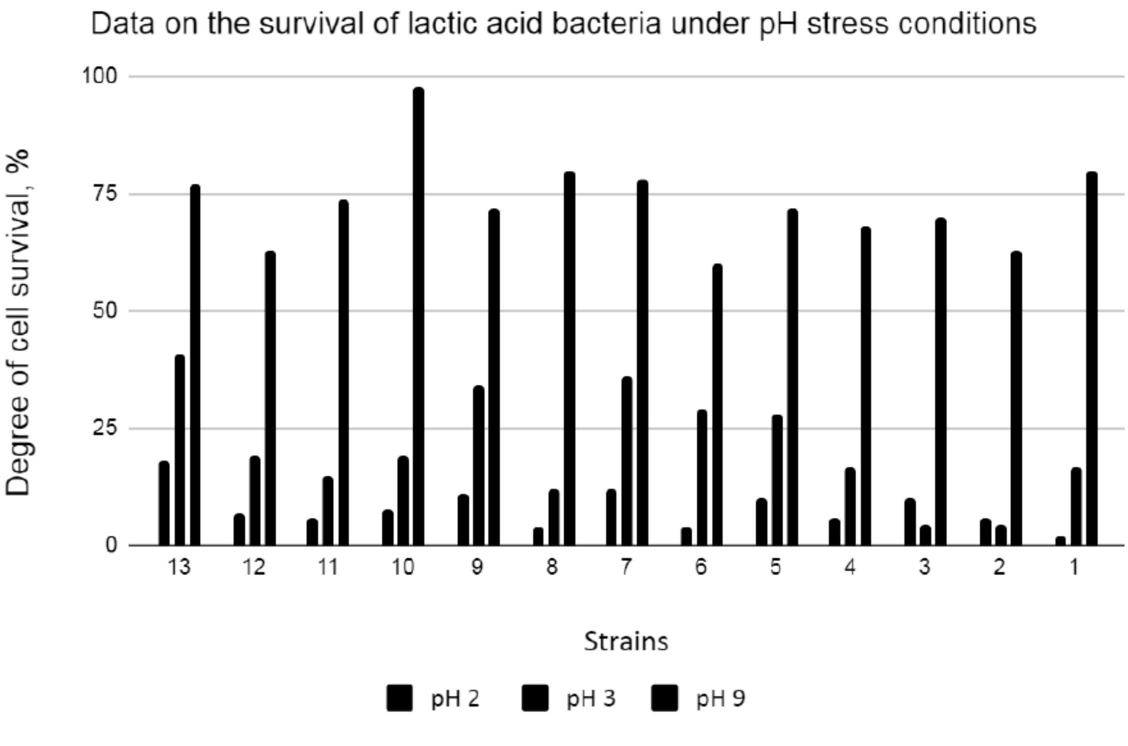

Figure 1. Change in lactic acid bacteria survival at different pH levels

At pH 2.0, lb. Cheese, Lmb. Plants and Lmb. Fermentation bacteria were marginally viable, with survival rates of 0.5-10%, 4-12% and 4-12% respectively, while Lbm. Acidophilus, Lbm. acidophilus were the most acid tolerant, with survival rates of 18% and 15% respectively.

Increasing the pH to 9.2 had no noticeable effect on the reactivity of the lactic acids analysed. Between 62% and 96% of the colonies exhibited an adsorptive reaction. Lmb. fermentum 4 (D) showed maximum compatibility with ester medium (96%).

The results showed that different species and strains of lactic acid bacteria responded quite differently to changes in pH, with Lmb. fermentum 3 (D) being the two most resistant to low pH. However, L.M.B. fermentum 4 (D) showed a significant alkaline medium reaction, which is very important for probiotic applications.

Conclusions. Thus, the experimental results showed that Lmb.casei 5, Lmb.plantarum 2 and Lmb.fertum 4 are more resistant to salt and bile than strains producing lactic acid bacteria. Lactobacillus strains can be used to predict how long they can retain their enzymatic activity when passing through the digestive system and settling in the intestine, as well as during storage in the refrigerator or on the counter. Therefore, by studying new strains of probiotic cultures used in the production of fermented dairy products and their ability to mimic different gastrointestinal conditions, suitable strains can be identified for use as probiotics in the production of functional foods. Probiotics can be useful not only for the prevention of infection, but also in the early stages of disease and during convalescence. Because the products are made from specific microorganisms and strains, they are of high quality. Adequate consumption of probiotic milk products can boost the immune system, improve intestinal microflora, act as a dietary supplement, have antibacterial effects in intestinal infections and lower cholesterol levels. The bacteria contained in probiotics determine the functional properties of the finished product. To be safe and effective, a probiotic product should contain a sufficient number of live active probiotic bacteria.

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НОВІ ШТАМИ ПРОБІОТИЧНОЇ КУЛЬТУРИ У ВИРОБНИЦІ МОЛОЧНИХ ФЕРМЕНТОВАНИХ ПРОДУКТАХ

Анотація

В останні роки зростає інтерес до продуктів, що приносять користь організму. Пробіотики, що містять живі штами природної мікробіоти людини є однією з найважливіших категорій функціональних продуктів харчування на сьогоднішній день. Пробіотики першого покоління, такі як «Bifidobacterium», «Lactobacillus» та «Propionibacterium», використовуються вже давно. Крім того, ці бактерії використовуються у виробництві різноманітних корисних молочних продуктів.

Індустрія функціональних продуктів харчування розширюється, оскільки дедалі більше людей хворіють, їх імунна система знижується. У зв'язку зі зменшенням корисної мікрофлори в організмі людини збільшується кількість патогенних та помірно патогенних мікроорганізмів та їх метаболітів. В результаті кишкова флора змінюється, що призводить до алергії, раку та анемії. Тому особливу увагу слід приділяти мікробному балансу шлунково-кишкового тракту, який необхідний людині для виживання, вживаючи органічні продукти, багаті на пробіотичну мікрофлору. Органічні функціональні продукти харчування з пробіотичною мікрофлорою – це розумний спосіб підтримки та покращення здоров'я людей. Стійкість штамів молочнокислих бактерій оцінювалася In vitro в умовах, наближених до функціонування травної системи людей. Виявили штами, які можна використовувати як пробіотики при виробництві продуктів здорового харчування.

Ключові слова: штами, пробіотики, бактерії, пробіотична культура, молочні продукти, ферменти, мікроорганізми.

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НОВЫЕ ШТАММЫ ПРОБИОТИЧЕСКОЙ КУЛЬТУРЫ В ПРОИЗВОДСТВЕ МОЛОЧНЫХ ФЕРМЕНТИРОВАННЫХ ПРОДУКТАХ

Аннотация

В последние годы растёт интерес к продуктам, которые приносят пользу организму. Пробиотики, содержащие живые штаммы естественной микробиоты человека, являются одной из наиболее важных категорий функциональных продуктов питания на сегодняшний день. Пробиотики первого поколения, такие как «Bifidobacterium», «Lactobacillus» и «Propionibacterium», используются уже давно. Кроме того, эти бактерии используются в производстве различных полезных молочных продуктов.

Индустрия функциональных продуктов питания расширяется, поскольку все больше людей заболевают, а их иммунная система снижается. В связи с уменьшением полезной микрофлоры в организме человека увеличивается количество патогенных и умеренно патогенных микроорганизмов и их метаболитов.

В результате кишечная флора изменяется, что приводит к аллергии, раку и анемии. Поэтому особое внимание следует уделять микробному балансу желудочно-кишечного тракта, который необходим человеку для выживания, употребляя органические продукты, богатые пробиотической микрофлорой. Органические функциональные продукты питания с пробиотической микрофлорой — это разумный способ поддержания и улучшения здоровья людей. Устойчивость штаммов молочнокислых бактерий оценивалась «In vitro» в условиях, приближенных к функционированию пищеварительной системы людей. Выявили штаммы, которые можно использовать в качестве пробиотиков при производстве продуктов здорового питания.

Ключевые слова: штаммы, пробиотики, бактерии, пробиотическая культура, молочные продукты, ферменты, микроорганизмы.