Assessment of lactic acid bacteria new consortia proteolytic activity

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Abstract. The paper presents data on the assessment of the proteolytic activity of new consortia of microorganisms containing functionally active strains of lactic acid bacteria. Lactobacillus in order to identify highly effective combinations for the production of fermented foods. The proteolytic activity was evaluated on a standard protein substrate albumin and casein, the main protein of milk and dairy products. As a result of the studies, consortia of lactic acid bacteria were identified containing strains of L. casei, L. fermentum, and L. plantarum in ratios of 1: 1: 1 and 2: 1: 1, exhibiting high proteolytic activity, on the basis of which they can be recommended for inclusion in the composition of starter cultures for the production of functional products.

1. Introduction

Lactic acid bacteria are the most studied and one of the most important groups of microorganisms used in the food industry, agriculture, medicine, veterinary medicine, etc. Lactic acid bacteria have various biotechnological properties and are of interest as an object of study for the development of functional food products, methods of correcting microecological disorders, as well as probiotic preparations for humans and animals.

Lactic acid bacteria are one of the generally accepted components that determine the ratio of a product to the category of functional food. In the scientific and technical literature today there are a sufficient number of reports on the beneficial effect of lactic acid microorganisms on the human body, including the ability to reduce the risk of malignant neoplasms, remove toxic substances and products of nitrogen metabolism from the body, help increase the absorption of calcium, and reduce cholesterol levels in blood, have an antibacterial effect on pathogenic microorganisms, etc. [1].

In addition to a wide range of positive biological effects on the human body, this group of microorganisms has unique biotechnological properties for the production of functional food products. The greatest interest of scientists around the world is attracted by antibiotic, gene-protective, antimutagenic, antioxidant properties of this group of microorganisms, as well as their ability to synthesize vitamins, enzymes, exopolysaccharides, bacteriocins and other biologically active substances [2-5].

Based on the analysis of the biotechnological properties of microorganisms all over the world, various versions of direct ferments are created, consisting of monocultures or their combinations; complex starter cultures of bifidobacteria and lactic acid bacteria with other groups of microorganisms, allowing to maximize the physiological, biochemical and technological potential of
microorganisms. Starter cultures (starter cultures), which include lactic acid bacteria, are traditionally used in the dairy industry (for the production of cheese, sour cream, yoghurt, fermented milk drinks, etc.), in baking (for the production of rye and rye-wheat breads), in the meat industry (in the technology of raw smoked and dry-cured meat products), for the production of pickled vegetables, fruits, fermented juices, sauces, etc. [6-8].

Lactic acid bacteria belonging to the genus Lactobacillus - lactobacillus and lactococcus, which are highly resistant to acids and salts and synthesize a wide range of biologically active compounds, are industrially valuable crops used in most starter cultures.

Among biologically active substances produced by lactic acid bacteria, proteolytic enzymes are of particular interest, which mainly form the texture, specific taste and aroma of fermented food products, and affect their biological value.

The proteolytic complex of lactic acid bacteria is represented by proteinases that provide the initial breakdown of proteins to peptides with the formation of a large number of oligopeptides; peptidases that break down peptides to amino acids and a transport system that carries out the transfer of proteolysis products across the cytoplasmic membrane of cells [9,10].

The transformation of proteins of food systems with the participation of lactic acid bacteria at the initial stages of proteolysis occurs under the influence of extracellular and cell-wall proteinases, which are monomeric serine proteinases with a molecular weight of 180-190 kDa. Further cleavage of peptides occurs under the influence of peptidases [9] of lactic acid microorganisms, which are metal-, serine- and cysteine peptidases with a molecular weight of 30 to 100 kDa.

The content and amount of a certain set of amino acids in fermented food products depends on the proteolytic ability of individual strains that make up the starter cultures, which largely determines the organoleptic and structural-mechanical properties of the product [11]. As a result of proteolysis, amino acids accumulate in fermented food products - valine, arginine, leucine, glutamic acid, proline, aspartic acid, leucine, lysine, etc., depending on the initial substrate and proteolytic activity of a particular strain of lactic acid bacteria.

An urgent and promising direction in the technology of bioproducts is the search and development of domestic competitive fermentations of lactic acid bacteria for functional food products with high biochemical activity and viability, possessing a number of functional and technological properties.

Since the properties of starter cultures substantially depend on the properties of specific strains, the consortia for the fermentation of food raw materials should include productively valuable crops, primarily showing high proteolytic activity. In the course of many years of work on the search for functionally active strains of lactic acid bacteria, we selected promising strains of lactic acid bacteria p. Lactobacillus with high biotechnological potential.

The aim of this work was to assess the proteolytic activity of new consortia of lactic acid bacteria containing functionally active strains of p. Lactobacillus.

2. Materials and methods

The objects of the study were consortia containing strains of lactic acid bacteria L. casei L. fermentum and L. plantarum in a ratio of 1: 1: 1; 1: 2: 2; 1: 1, and 1: 2: 1. The temperature optimum of the strains lies in the range of 30-37 °C, the strains reproduce well in a wide temperature range from 15 to 45 °C, the optimum pH value is 5.5-6.2, the strains are resistant to alkaline medium reactions (pH 9.2), able to grow in an acidic (pH = 3) environment (table 1).

These strains are distinguished by high proteolytic activity [12], exhibit antioxidant [13], gene-protective and antimutagenic properties [14], actively suppress the growth of sanitary-indicative microflora of food products [15], which confirms their potential for inclusion in a consortium intended for functional food products.
Table 1. Characterization of the strains that are part of the consortia.

| Strain       | Microscopic                                                                 | Growth at temperature, °C | Growth with NaCl, % | Growth at pH |
|--------------|------------------------------------------------------------------------------|---------------------------|---------------------|--------------|
| *L. plantarum* 21  | Straight rods, arranged in pairs or in short chains                          | +                         | +                   | +            |
| *L. casei* 32   | Thick rods of medium length single and in short chains                        | +                         | +                   | +            |
| *L. fermentum* 12 | Medium-length rods, located mainly in chains                                 | +                         | +                   | -            |

The study of the activity of lactic acid bacteria starter cultures was carried out by recording absorption spectra in the wavelength range of 220-280 nm according to the method of Warburg and Christian [16], as well as according to the method [17], the principle of which is to measure the amount of bacterial proteolysis products not precipitated by trichloroacetic acid. 1% solutions of serum albumin and casein were used as model substrates.

3. Results and discussion

All the strains under study have an intense proteolytic effect on the model substrate containing 1% serum albumin solution [12], however, the degree of protein hydrolysis by the microorganism consortium may differ significantly from that of the original strain, since it is determined not only by the properties of a particular microorganism, but also by their interaction during co-cultivation.

To identify the proteolytic activity of the consortia of microorganisms under consideration, we carried out studies to assess the content of proteins in model substrates after their fermentation with various ratios of the studied microorganisms.

The results of the study of the proteolytic activity of the consortia of the studied strains showed that during the fermentation of casein, there was an intense decrease in the protein content relative to the control values, and the most pronounced changes were characteristic of samples containing predominantly the *L.casei* strain (2: 1: 1) and an equal number of viable cells *L. casei, L. fermentum*, and *L. plantarum* (1: 1: 1). For these consortia of lactic acid bacteria, the degree of casein hydrolysis was 83.4% and 79.2%, respectively (figure 1).

![Figure 1. Degree of protein hydrolysis by lactic acid bacteria consortia.](image)
Similar results were obtained when evaluating the proteolytic activity of the studied consortia of lactic acid bacteria during albumin fermentation, however, the ability of these consortia to hydrolyse this protein is lower by an average of 15% compared to the degree of proteolysis demonstrated on casein.

The least ability to hydrolyse proteins was demonstrated by a consortium of microorganisms in a culture ratio of 1: 1: 2, containing a large amount of L. plantarum strain; the degree of hydrolysis of casein and albumin by this consortium was 56.5 and 37.6%, respectively.

Thus, the analysis of the data obtained on the study of the effect of lactic acid bacteria starter cultures on the content of hydrolysis products in model protein systems indicate a high proteolytic ability of the consortia of L. casei, L. fermentum, and L. plantarum cultures when the strains are combined in ratios of 1: 1: 1 and 2: 1: 1, and these starter cultures of lactic acid bacteria effectively affect not only casein, but also albumin.

The results of research work will further serve for the development of new starter cultures based on lactic acid bacteria p. Lactobacillus for fermented functional food products on meat and dairy basis, will allow planning the component composition of products based on knowledge of the level of proteolytic activity of the consortia of lactic acid bacteria used and the characteristics of protein hydrolysis.

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