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CULTIVATION OF LACTIC ACID BACTERIA ON NUTRIENT MEDIA CONTAINING SOY MOLASSES

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Abstract. *One of the by-products of the soybean processing is soy molasses. Due to the lack of effective disposal methods, soy molasses often ends up in landfills or is used as liquid fertilizers, which can lead to environmental pollution. At the same time, soy molasses contains a significant amount of carbohydrates, vitamins and minerals, which can contribute to the effective growth and reproduction of microorganisms, the accumulation of high concentrations of biomass and metabolic products. The purpose of this work is to evaluate the ability of lactic acid bacteria to grow on nutrient media containing soy molasses. The conducted studies allow us to conclude that lactic acid bacteria of different taxonomic groups (representatives of the genus Lactiplantibacillus, Lacticaseibacillus, Levilactobacillus, Lentilactobacillus,*

Limosilactobacillus, Loigolactobacillus, Pediococcus, Leuconostoc, Weissella) grow quite actively, accumulate biomass and acidic metabolic products on media containing soy molasses (2.5–5.0%), mainly on the medium, containing 5% soy molasses. In the context of growing interest in environmentally friendly and cost-effective production methods, the processing of soy waste, including soy molasses, into valuable products during lactic acid fermentation is a promising direction.

Keywords: *lactic acid bacteria, soy molasses, cultivation, biomass, acid production.*

Introduction. Lactic acid bacteria are widespread in nature, play an important role in such fields as the food industry, agriculture, veterinary medicine, medicine, etc., and are convenient model objects for metabolic, environmental, and genetic research [1–4]. The high growth rate and metabolic activity of these microorganisms are essential conditions for their successful practical application.

Cultivation is the main stage of any biotechnological production and largely determines the quantitative and qualitative characteristics of the final product. Among non–pathogenic microorganisms, lactic acid bacteria are the most demanding in terms of nutrient content in the cultural medium and require, in addition to fermentable carbohydrates, the presence of amino acids, purines, pyrimidines, vitamins, etc. The cultivation of these bacteria is carried out on complex, expensive nutrient media containing relatively large amounts of yeast extract, peptone, etc. [1, 5–7]. In this regard, research aimed at finding inexpensive nutrient media and their ingredients for the cultivation of lactic acid bacteria is relevant.

The problem of expanding the resource capabilities of the food and processing industry can be solved through the use of industrial waste. One of the by-products of the soy processing process, especially in the production of soy protein concentrate, is soy molasses. It is formed during the production of soy protein concentrate by the traditional method of water-alcohol washing of soy "white petal". Depending on the processing technology used, the volume of soy molasses output may vary, but the average yield is about 232 kg per ton of processed soybean meal. Due to the lack of effective disposal methods, soy molasses often ends up in landfills or is used as liquid fertilizers, which can lead to environmental pollution. At the same time, soy molasses contains a significant amount of carbohydrates, vitamins and minerals, which can contribute to the effective growth and reproduction of lactic acid bacteria, the accumulation of high concentrations of biomass and acidic metabolic products during fermentation [8–13].

Soy molasses is a thick dark brown liquid containing from 45% to 70% of solids. Molasses contains various components such as carbohydrates, phospholipids, proteins and minerals. Carbohydrates make up the balk of the solids of soy molasses, accounting for 75% to 80% of the total. Phospholipids – from 9% to 12%, low molecular weight proteins – from 3% to 6%, and minerals – from 5% to 7%. Carbohydrates are dominated by monosaccharides, which make up 65% of the composition, as well as oligosaccharides such as raffinose (5–7 %) and stachyose (30–32 %). In addition, molasses contains isoflavonoids and saponins derived from soy.

An important advantage of using soy molasses for the cultivation of lactic acid bacteria is economic efficiency, since soy waste, including molasses, is a low-cost raw material, which reduces the overall cost of producing the final product. The use of soy molasses for the cultivation of lactic acid bacteria not only contributes to economic benefits, but also supports the principles of sustainable development, sustainable use of resources, reduction of the ecological footprint, helps in solving the problem of waste disposal in the agricultural sector.

It can be concluded that in the context of growing interest in environmentally friendly and cost-effective production methods, the processing of soy waste, including soy molasses, into valuable products during lactic acid fermentation is becoming an urgent task. In this regard, the purpose of this work is to evaluate the ability of representatives of different taxonomic groups of lactic acid bacteria to grow on nutrient media containing soy molasses.

The objectives of the study included:

1. To evaluate the accumulation of biomass of lactic acid bacteria in standard media and nutrient media containing soy molasses;

2. Evaluate the level of acid formation of lactic acid bacteria in standard media and nutrient media containing soy molasses.

Materials and methods. The object of the study were 18 strains of representatives of homo- and heterofermentative lactic acid bacteria of the species *Lactiplantibacillus plantarum*, *Lactiplantibacillus paraplantarum*, *Lacticaseibacillus casei*, *Pediococcus pentosaceus*, *Levilactobacillus brevis*, *Lentilactobacillus buchneri*, *Limosilactobacillus fermentum*, *Leuconostoc mesenteroides*, *Loigolactobacillus coryniformis*, *Weissella cibaria*.

The characteristics of the soy molasses used for the cultivation of lactic acid bacteria are given in the table.

Table – Characteristics of soy molasses

Mass fraction of solids, %	not less than 75.0
Mass fraction of sucrose by direct polarization, %	58–65
The mass fraction of crude protein converted in abs. dry matter, %	not less than 5,0
Mass fraction of crude fat converted in abs. dry matter, %	not less than 7,0

Soy molasses was diluted in distilled water at three concentrations of 5.0%, 3.5%, and 2.5%. After that, it was inoculated with 18 strains of lactic acid bacteria and incubated for 48 hours. The comparison medium was the MRS medium [14], in which the tested cultures were also grown for 48 hours. The accumulation of biomass during the growth of lactic acid bacteria on media of different compositions was monitored by the change in the optical density of the cultural liquid at a wavelength of 590 nm. The acid formation activity of lactic acid bacteria was assessed by the level of active acidity (pH) and titrated acidity, which was expressed in degrees of Turner (^{o}T).

Data processing was carried out using the Microsoft Excel 2016 analysis package.

Results and discussion. Complex, expensive nutrient media containing relatively large amounts of yeast extract, peptone, etc. are used for the cultivation of lactic acid bacteria. The MRS medium is the standard and widely used medium for the maintenance of various groups of lactic acid bacteria [14]. Soy molasses is a natural, inexpensive byproduct of soy protein production. Taking this into account, a comparative study of the growth and accumulation of acidic metabolic products by lactic acid bacteria of different taxonomic groups in the MRS medium and media containing 5.0%, 3.5%, 2.5% soy molasses was carried out.

It has been revealed that homo- and heterofermentative lactic acid bacteria are able to grow and accumulate biomass on media containing soy molasses. The conducted studies have shown that lactic acid bacteria grow best on MRS medium and soy molasses with a concentration of 5%. Among homo- and heterofermentative lactic acid bacterial, cultures (representatives of the species *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Loigolactobacillus coryniformis*, *Pediococcus pentosaceus*, *Levilactobacillus brevis*, *Weissella cibaria*, *Leuconostoc mesenteroides*) have been identified, which shoved a level of biomass accumulation during cultivation on media containing 5.0–3.5% soy molasses exceeding the desired value on the MRS medium.

One of the main characteristics of lactic acid bacteria is their acid formation activity. It was found that for all the lactic acid bacteria studied, the titrated acidity index was higher when cultured in MRS medium than in media containing soy molasses. Homofermentative bacteria were characterized by higher values of titrated acidity of the cultural fluid (112–150^{o}T) compared with heterofermentative lactic acid bacteria (68–90^{o}T). When cultivated in media containing soy molasses, the titrated acidity index was lower than in the MRS medium. In homofermentative bacteria, the titrated acidity ranged from 22 to 85 ^{o}T, in heterofermentative lactic acid bacteria – from 10 to 71 ^{o}T. At the same time, the index of titrated acidity is higher in lactic acid bacteria cultivated in soy molasses by 5%.

It was found that, in contrast to the values of titrated acidity, the indicators of the active acidity of the culture fluids of the studied lactic acid bacteria grown in MRS medium and in media with soy molasses differed less significantly (by 13–19%). In some representatives of *Loigolactobacillus coryniformis*, *Pediococcus pentosaceus* and *Levilactobacillus brevis* species, the level of active acidity of the cultural liquid was higher in media with soy molasses than in the standard MRS medium.

Conclusion. The conducted studies allow us to conclude that lactic acid bacteria of different taxonomic groups (representatives of the genus *Lactiplantibacillus*, *Lacticaseibacillus*, *Levilactobacillus*, *Lentilactobacillus*, *Limosilactobacillus*, *Loigolactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella*) grow quite actively and accumulate acidic metabolic products on media containing soy molasses (2.5–5.0%), mainly on an environment containing 5.0% soy molasses.

In the context of growing interest in environmentally friendly and cost-effective production methods, the processing of soy waste, including soy molasses, into valuable products during lactic acid fermentation is a promising direction.

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