Jerusalem artichoke tubers for producing vegetable probiotic functional beverages with lactic acid bacteria

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Abstract. The lactobacilli (L. plantarum, L. bulgaricus, L. salivarius, and L. rhamnosus) growth in water extracts of Jerusalem artichoke tubers (solvent ratio of 1:10) with solid particles was studied. The cultures were found to have great specific growth rates and high abundance (up to 10.3 log (CFU/ml)) for 6 hours. The adhesion of the cells to the tuber particles was revealed by a modified counting technique and confirmed by scanning electron microscopy. Lactobacilli were shown to be able to maintain the abundance required (not less than 7.5 log (CFU/ml)) at 2–6 °C for 28 days, which was presumably influenced by the adhesion of the cells to the tuber particles. The data obtained allowed claiming that water extracts of Jerusalem artichoke tubers are promising for production of probiotic functional beverages with lactic acid bacteria.

1. Introduction
Functional products are foods that have good nutritional properties and positive effect on health at regular consumption [1]. They are obtained from their traditional analogues enriched with biologically active components (vitamins, dietary fiber, probiotics, antioxidants, etc.) that can improve health or reduce risks of diseases. These products occupy a certain market segment [2, 3]. Probiotics are living microorganisms that in adequate amounts benefit the host organism [4]. This means that, taking into account the daily intake rate, microorganisms in a sufficient amount of more than 10^9 cells must be present in food [5]. For each product, the minimum daily intake dose required to achieve a positive effect should be indicated [4]. Lactobacilli are gram-positive, optionally anaerobic, usually immobile, and non-spore-forming bacteria. Their characteristic feature is the ability to carry out lactic acid fermentation [6, 7, and 8].

Traditionally, milk is the basis for a wide range of probiotic foods. However, beverages that do not contain dairy components occupy one of the largest sectors in the functional food market that can increase due to research studies aimed at selecting new raw materials that have not been used in industrial production yet [9, 10]. Reengineering of products and processes is performed in order to meet the needs of people with hypolactasia (lactose intolerance) and vegetarian preferences for new nutritious probiotic beverages [11, 12].

Jerusalem artichoke (Helianthus tuberosus L) is a tuberous plant with high nutritional value that is mainly associated with carbohydrates, including inulin at a fairly high concentration (about 16%) [13, 14]. When consumed, inulin is not digested in the upper digestive tract and is metabolized by the autochthonous colon microflora. Therefore, it belongs to prebiotics and can affect both the composition and the activity of the microflora of the gastrointestinal tract, thus exerting a healing effect on the host’s
body [15, 16]. This plant also has several other advantages. Its tuber have a relatively high protein content (2%). Jerusalem artichoke proteins contain an increased amount of glutamic and aspartic acids. They are closely related to carbohydrate metabolism through the tricarboxylic acid cycle that is a source of macroergic bonds [17, 18].

Studies of the probiotic microorganisms growth on plant substrates make it possible to select optimal cultivation regimes, study nutrient requirements of microorganisms (carbon sources, amino acids, peptides, etc.), and draw conclusions on the application perspectiveness of the specific substrate [19, 20].

The purpose of the work was to study the effect of the plant substrate particles (Jerusalem artichoke tubers) on the lactobacilli growth in submerged culture and its storage stability. The obtained data can be used in the development of a functional beverage.

2. Materials and methods

2.1. Microorganism and cultivation conditions

We studied bacterial strains of Lactobacillus obtained from the State Research Institute of Genetics and Selection of Industrial Microorganisms of the National Research Center, i.e. L. plantarum (ACIM B-7583), L. delbrueckii subsp. bulgaricus (ACIM B-4626), L. salivarius (ACIM B-2214), and L. rhamnosus (ACIM B-8238). These Lactobacilli belong to the most important representatives of the human intestinal microbiota.

Lactobacilli were cultured, and the inoculum was obtained in MRS medium (with some modifications) of the following composition (g/L): 2 of Na₂HPO₄×12H₂O; 5 of CH₃COONa×3H₂O; 2 of ammonium citrate; 0.2 of MgSO₄×7H₂O; 0.05 of MnSO₄×4H₂O; 10 of peptone; 10 of meat extract; 5 of yeast extract (YE) (Difco, USA); 20 of glucose; and up to 1 L of distilled water, pH 7.0. To determine the count of viable cells, the MRS medium was prepared and added with agar in an amount of 10 g/L [21]. The inoculum was obtained by incubating lactobacilli at 37 °C for 24 h (this time period corresponded to the late logarithmic growth phase) [22].

2.2. Plant raw material

Crushed tubers of Jerusalem artichoke—its particles were suggested as carriers for the lactobacillus adhesion—were used as a source of nutrients in the experimental medium to obtain a functional beverage. To prepare a nutrient medium, Jerusalem artichoke tubers previously washed and ground (0.5–1 mm) were mixed with water in a ratio of 1:10 [23]. The extraction was carried out simultaneously with sterilization at a temperature of 115 °C for 30 min. No solid precipitate was separated and no additional nutrient sources were added. The medium was inoculated with a daily culture in an amount of 2% of the medium volume at 37 °C for 12 h. After cultivation, the samples were stored at a temperature of 2–6 °C. In a series of experiments, the stability of various cultures of lactobacilli grown in extracts from crushed Jerusalem artichoke tubers was studied during storage for 28 days. The experiments were performed in triplicate.

2.3. Determining the count of microorganisms

The microorganisms were counted by the technique of ten-fold serial dilutions with inoculation on a solid MRS medium. Before inoculation on a solid nutrient medium, the suspension was additionally (a modified technique) treated with sterile phosphate buffer of the following composition (g/L): 8 of NaCl; 0.24 of Na₂HPO₄; 0.2 of KCl; and 0.24 of KH₂PO₄. For this, carefully mixed samples (1 mL) were placed in Eppendorf tubes and centrifuged (Eppendorf Centrifuge 5417C, 7000 rpm, 10 min); the supernatant was decanted. Then, it was added to the precipitate of 1 mL of sterile buffer solution, mixed, and incubated for 30 min at 37 °C. Centrifugation was repeated with decantation of the supernatant. Then, the buffer solution was added to the tubes to a total volume of 1 mL. To determine the titratable acidity, 10 mL of the sample was mixed with 20 mL of distilled water, and potentiometric titration of 0.1 N was performed with sodium hydroxide solution to a pH of 8.80.
2.4. Scanning Electron Microscopy
The lactobacilli adhesion was studied with respect to Jerusalem artichoke particles, using scanning electron microscopy (SEM) [24, 25]. After fermentation, the suspension was centrifuged (7000 rpm, 10 min), and the supernatant was decanted. Cells were fixed with a 5% glutaraldehyde solution in HEPES [N-(2-hydroxyethyl) piperazin-N'-(2-ethanesulfonic acid)] buffer (100 mM, pH of 7.4) for 30 min, washed twice in phosphate buffer, dehydrated in ethanol in stages (35%, 50%, 75%, 95%, and 100%), and left overnight to dry in a desiccator under vacuum. Then, the samples were attached using double-sided adhesive tape to the cork and covered with platinum (layer thickness of 8 nm). To visualize the preparations, a JEOL 1610LV scanning electron microscope (JEOL, Japan) with an energy-dispersive spectrometer for electron probe microanalysis SSD X-Max Inca Energy (Oxford Instruments, United Kingdom) was applied.

2.5. Statistical processing
The data presented in the graphs and tables are the arithmetic mean of three parallel enumerations. One-way analysis of variance to confirm statistical significance of the results of determining the lactobacilli count by the standard and modified techniques was carried out at a maximum error of 0.05; 0.025; and 0.01, using MS Excel 2013. The variance was checked according to the Fisher criterion.

3. Results and discussion

3.1. Growth of lactobacilli in aqueous extracts of Jerusalem artichoke tubers
To obtain experimental fermented beverages based on Jerusalem artichoke tubers, lactobacilli were cultured in extracts obtained without solid phase separated. All samples showed a high growth rate and an intense pH decrease that continued after the growth arrest, which, presumably, is associated with the need for cells to receive energy for maintenance. We also observed a difference between the lactobacilli counts determined by standard and modified techniques on average 7 times, which can be associated with the destruction of agglomerates formed during cultivation. The study found no lag phase of L. delbrueckii subsp. bulgaricus (figure 1a) and L. rhamnosus (figure 1b); and the L. plantarum (figure 1c) and L. salivarius (figure 1d) lag phase was 1 hour. Specific growth rates were 0.51–0.52 h⁻¹ for L. delbrueckii subsp. bulgaricus and L. rhamnosus and 0.58–0.59 h⁻¹ for L. plantarum and L. salivarius in the exponential phase. The results obtained suggest that Jerusalem artichoke tubers extract is a favorable medium for the growth of the lactobacilli strains studied. At the end of fermentation, there was determined titratable (active) acidity that was within 100–101 °T in all samples, which corresponded to products with lactic acid bacteria.

The obtained results are generally consistent with those presented in the literature. When cultivated on complex carbohydrates of L. plantarum [26] and during fermentation of Jerusalem artichoke juice [27], a two-stage growth was observed. After 24 h of fermentation of L. plantarum in a rice extract at various inulin concentrations, the abundance was 2.7×10⁶ CFU/mL [28]. The growth of L. plantarum in pineapple juice showed that after 24 hours the count of viable microorganisms overcame the level of 5×10⁹ CFU/mL [29]. Studies of a probiotic product based on cabbage juice added with L. rhamnosus culture showed that the lactobacilli count after fermentation did not exceed 9 log CFU/mL [30]. The analysis of the data during the cultivation of lactobacilli on dairy whey products added with plant components (celandine, barberry, and alfalfa) showed that after 24 hours of fermentation the abundance averaged 9-10 log CFU/mL [31]. Thus, water extracts of Jerusalem artichoke tubers are a favorable substrate for the lactobacilli growth, and in some cases their count was higher than that on other plant substrates.

3.2. Stability of fermented beverages during storage
To confirm the stability of lactic acid bacteria in the extract after cultivation, we studied the dynamics of their counts during storage for 28 days at a temperature of 2–6 °C. The variance analysis found that in most cases the differences between the results obtained by the standard and modified techniques (table
were significant at a prescribed error of not more than 0.025 that is an acceptable significance in biological studies.

**Figure 1.** Growth curve in Jerusalem artichoke tuber extract determined by standard and modified techniques and the pH dynamics during cultivation, where a) is L. delbrueckii subsp. Bulgaricus; b) is L. rhamnosus; c) is L. plantarum; and d) is L. salivarius.

**Table 1.** Dynamics of the lactobacilli count in a fermented beverage determined by standard and modified techniques during storage at a temperature of 2-6 °C for 28 days.

| Culture        | 0 day | 7 days | 14 days | 21 days | 28 days |
|----------------|-------|--------|---------|---------|---------|
|                | Standard | Modified | Standard | Modified | Standard | Modified | Standard | Modified | Standard | Modified |
| L. plantarum   | 8.45±  | 9.46±   | 8.24±   | 9.30±   | 8.02±   | 9.34±   | 7.88±   | 9.00±   | 8.00±   | 8.64±   |
|                | 0.07b  | 0.03b   | 0.03b   | 0.00b   | 0.04b   | 0.00b   | 0.01b   | 0.03b   | 0.00b   | 0.01b   |
| L. bulgaricus  | 9.58±  | 10.40±  | 8.63±   | 9.54±   | 8.29±   | 9.25±   | 7.83±   | 8.81±   | 7.52±   | 8.49±   |
|                | 0.05b  | 0.05b   | 0.01b   | 0.01b   | 0.03b   | 0.02b   | 0.01b   | 0.00b   | 0.03b   | 0.04b   |
| L. salivarius  | 9.72±  | 10.04±  | 9.13±   | 9.97±   | 8.71±   | 9.52±   | 8.56±   | 9.29±   | 7.88±   | 8.87±   |
|                | 0.01b  | 0.05b   | 0.03b   | 0.02b   | 0.01b   | 0.03b   | 0.01b   | 0.04b   | 0.02b   | 0.04b   |
| L. rhamnosus   | 9.32±  | 9.49±   | 9.08±   | 9.18±   | 8.48±   | 9.12±   | 8.41±   | 9.03±   | 8.41±   | 9.03±   |
|                | 0.05b  | 0.05b   | 0.06b   | 0.08b   | 0.01b   | 0.03b   | 0.04b   | 0.04b   | 0.03b   | 0.10b   |

* Significance is when a: p < 0.05; b: p < 0.025; and c: p < 0.001
3.3. Adhesion of lactobacilli to Jerusalem artichoke particles

In all cases, we observed the lactobacilli increasing their count at the applied modified technique compared to the standard one, which presumably is associated with their agglomeration that is also possible on the surface of Jerusalem artichoke particles (coadhesion) [32]. Bacteria often form bonds through electrostatic and van der Waals interactions. The adhesive behavior of microbial cells depends on the combination of these processes and on the hydrophobic nature of the surfaces involved [33]. Zeta potentials [34] and the formation of extracellular substances on or outside the cell surface also have an effect [32, 35]. The data on the stability of probiotic microorganisms during storage of the probiotic beverage in a refrigerator are not inferior to those in previous studies that used Jerusalem artichoke juice [29].

The increase in the bacteria count determined by the modified technique compared to the standard one was not the same for different cultures. So, immediately after incubation of lactobacilli *L. plantarum*, these values differed 10.2 times and after 14 days of storage 20.9 times. Differences in the determination of *L. bulgaricus* count also ranged from 6.6 to 9.6 times. On the other hand, in the study of *L. rhamnosus*, they did not exceed 4.4 times. It can be assumed that the first two cultures are characterized by a greater degree of agglomeration. Perhaps this is due to different levels of osmotolerance in microorganisms [32], or increased extracellular conductivity in *L. plantarum* and *L. bulgaricus*, which is a consequence of greater membrane permeability [7].

The SEM studies (figure 2) showed the agglomeration of *L. plantarum* cells of different densities on the surface of Jerusalem artichoke particles. The microcolonies developed on the surface of the carrier were one of the stages of the biofilm that followed the fixing of plankton cells on the surface and its division [36]. The density of the bacteria colonization of particles was not the same in different areas, i.e. in some areas, cells formed dense clusters in several layers. These structures can be attributed to the areas of the biofilm formed. However, areas with single cells or single microcolonies predominated. Colonization of Jerusalem artichoke particles can contribute to increased viability of sells during the intestinal passage, thereby increasing the value of the finished product [34, 36].

![Figure 2. Scanning electron microscopy of particles of crushed Jerusalem artichoke tubers with agitated *L. plantarum* cells, magnification ×5,000.](image)

The viable cell count was not lower than $10^8$ CFU/mL when determined by the modified technique after 28 days of storage and not lower than $3 \times 10^7$ CFU/mL when determined by the standard one. In accordance with the Technical Regulation of the Customs Union 021/2011, at the end of the shelf life of sour-milk and other fermented products, the lactobacilli count should be at least $10^7$ CFU/mL [38]; thus, the products obtained in this study were characterized by high quality indicators. The proposed techniques can be applied not only in food production, but also in production of feed additives and in industrial biotechnology.
4. Conclusion
Analyzing the data on the viable cell count in probiotic products, containing solid particles, we should note that the results obtained by the modified technique were closer to the true values. The proposed technique can be a useful tool for the quality control of food products, containing live probiotic microorganisms, which, however, requires further research.

Probiotic Lactobacillus bacteria are characterized by high growth rates on extracts of Jerusalem artichoke tubers even without preliminary treatment (for example, enzymatic or acid hydrolysis), as well as without additives. The lack of sucrose in the beverage developed will attract potential consumers, since it will become appropriate for people suffering from diabetes [25]. Inulin in the extract known as a prebiotic allows attributing the product to synbiotic. However, these properties should be confirmed by additional research.

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