UDC: [57.083.1:579.864]:664.2-027.33

PROSPECTS OF USING BY-PRODUCTS OF POTATO STARCH PRODUCTION AS COMPONENTS OF GROWTH MEDIA FOR LACTIC ACID BACTERIA CULTIVATION

A. Bezusov, Doctor of Technical Sciences, Professor
T. Afanasieva, PhD**,** Associate Professor
N. Dotsenko, PhD**,** Associate Professor
H. Palvashova, PhD**,** Associate Professor
Department of Bioengineering and water
Odessa National Academy of Food Technologies
112, Kanatna Str., Odessa, Ukraine, 65039

DOI: https://doi.org/10.15673/fst.v14i3.1796

Article history

Received 18.05.2020
Reviewed 24.06.2020
Revised 10.08.2020
Approved 17.09.2020

Correspondence:

T. Afanasieva
E-mail: tana79.a@gmail.com

Cite as Vancuver style citation

Bezusov A, Afanasieva T, Dotsenko N, Palvashova G. Prospects of using by-products of potato starch production as components of growth media for lactic acid bacteria. Food science and technology. 2020;14(3):13-20. DOI: https://doi.org/10.15673/fst.v14i3.1796

Цитування згідно ДСТУ 8302:2015

Prospects of using by-products of potato starch production as components of growth media for lactic acid bacteria / Bezusov A.T., et al // Food science and technology. 2020. Vol. 14, Issue 3. P. 13-20 DOI: https://doi.org/10.15673/fst.v14i3.1796

Abstract. The paper is concerned with the search for inexpensive alternative components of substrates of culture media for lactic acid bacteria. To be used in the food industry more extensively, lactobacilli should be produced in larger volumes. Bacteria-growing enterprises and those manufacturing probiotic preparations are interested in non-traditional components of growth media. However, it is quite a problem to create a cheap growth medium, because lactobacilli are very demanding of the content of certain components. Traditional culture media can include up to 40 components (peptones, yeast and vegetable extracts, vitamins, etc.). The research has proved that waste and by-products of potato starch production (potato juice and juice-containing water) can be used as components of a substrate to culture lactobacilli on. The strain used in the research was *Lactobacillus plantarum 8R-A3*. The growth kinetics of lactic acid bacteria has been analysed on different vegetable extracts. Tomato and cabbage extracts were used as traditional components of synthetic media to compare with. The findings have shown that on a substrate from by-products of potato starch production, after 96 hours of culturing at 37°C, the accumulation of *Lactobacillus plantarum* biomass is 20g/l (on juice-containing water) and 25g/l (on potato juice). Accumulation of bacteria wet weight has also been confirmed by determining the kinematic viscosity of the culture fluid. This parameter for potato juice after thermostatting was 6.77mm^2/s, which was somewhat higher than the viscosity of the tomato and cabbage extracts. It has been proved that by-products of potato processing ensure sufficient growth of lactic acid bacteria biomass. On the fourth day, the number of bacteria was $3 \cdot 10^8$CFU/cm^3. It has been shown how practical it is to use potato processing by-products as growth media substrates in culturing lactic acid bacteria *Lactobacillus plantarum*.

Key words: growth media, culturing, lactic acid bacteria, *Lactobacillus plantarum*, food waste, starch production.

Introduction. Formulation of the problem

People have used lactic acid bacteria (LAB) since prehistoric times to make different foods. The progress of agricultural microbiology and biotechnology has allowed a wider range and a higher level of applications of these microorganisms. In the food industry, lactic acid bacteria are traditionally used to make fermented dairy products, to bake bread, to preserve fruit and vegetables (sauerkraut, pickled cucumbers, olives in brine), to obtain fermented sausages and dried fish. Under certain conditions, lactobacilli can synthesise various bioactive substances: organic acids, vitamins, enzymes, etc. In agriculture, lactic acid bacteria are very important in animal feed preservation. For the above reasons, the lactobacilli biomass market is but growing with years.

Lactic acid bacteria belong to the heterogenic group of microorganisms. They include such genera as *Lactobacillus*, *Streptococcus*, *Pediococcus*, and *Leuconostoc*. Various species of bacteria, yet even strains within the same species, can be quite different from each other in their physiology and metabolic features. So it is but natural to culture them using different growth media. Creating a proper growth media for LAB is a topical and difficult task. There is no universally applicable selective medium to culture all types of lactic acid bacteria. That is why MRS agar, a classic medium for lactobacilli (the abbreviation stands for *de Man, Rogosa & Sharpe*) [1], can be modified by changing the pH value, the concentration of inhibiting agents, the duration and temperature of culturing. However, it is a very hard task to create a medium for LAB. Besides, compositions of culture media often contain components that increase their

cost. For large-scale production, one should search for the optimal culture medium that requires small amounts of raw materials. So today's topical problem is finding and manufacturing cheap components of growth media for culturing lactic acid bacteria. A good alternative can be plant-derived components that would allow developing new growth media for LAB and improving the existing ones.

Analysis of recent research and publications

Lactic acid bacteria are genetically diverse bacteria that produce lactic acid, the main product of their metabolism. They are gram-positive, catalase and oxidase-negative bacteria. They never sporulate, move spontaneously, and grow under anaerobic conditions [2].

Lactic acid bacteria are known as auxotrophic organisms, so they are very fastidious about artificial growth media. For successful culturing of a certain species of microorganisms, its growth medium should be as close to the natural conditions of this organism's existence and growth as possible. To culture bacteria of the genus *Lactobacillus*, media are used that are rich in nutrients (yeast extract, skimmed milk hydrolysate, peptones, Tween 80) and in various salts (including sodium acetate), and have a low pH (4.5–6.2) [3-7]. For their growth, most lactic acid bacteria need organic nitrogen forms they cannot synthesise themselves (such as soya peptones, peptones of animal origin) [7-9]. A lot of LAB species in their development depend on vitamins (especially of the B group) [5,6]. This is why different extracts (like those from yeast or maize) [8] and some other substances added to a culture media have so big an effect on the growth of these organisms. Usually, to culture *Lactobacillus* bacteria, carbohydrates that contain reducing agents are used as the source of carbon, because lactic acid bacteria do not normally exhibit amylolytic activity and cannot consume polysaccharides. With glucose as the source of carbon, substrate conversion is quite high (normally 95–98%). There are studies proving that to culture lactic acid bacteria, glucose can be replaced with different hydrolysates and extracts of both plant and animal origin [9].

Of all special growth media, the most commonly used is MRS agar [1], which is rich in nutrients and growth factors. It contains extracts from yeast and beef, glucose, peptones, sodium acetate, ammonium citrate, and Tween 80 (the source of fatty acids for the metabolism of lactobacilli). Table 1 presents the medium's components and their quantities, its pH being 6.2–6.4 [10].

MRS agar also proves useful when working with probiotic lactobacilli, in particular, to isolate these microorganisms from food products and natural biotopes. However, this medium has some basic disadvantages. One of them is its too complex composition; another drawback is a number of components it includes too expensive to use on an industrial scale [11-13].

Table 1 – Composition of the growth medium MRS agar [10]

Components	g/l
Yeast autolysate	5
Peptone	10
Glucose	20
Ammonium citrate	2
Sodium acetate	5
Proteose peptone	10
$MgSO_4 \cdot 7H_2O$	0.1
$MnSO_4 \cdot 4H_2O$	0.05
K_2HPO_4	2
Tween 80	1
Agar-agar	12

A review of publications on the topic has shown that lactobacilli are demanding of the presence of different nitrogen sources in a growth medium [14]. Traditionally, these are peptones, yeast extracts, by-products of food industries, and agricultural waste. However, they are quite expensive and make so the media they are in. That is why it is so topical a task to find alternative components to culture lactic acid bacteria.

A by-product resulting from manufacture of dairy foods (like cheese) is milk whey. It is rich in lactose, soluble proteins, lipids, and minerals, and retains about 55% of the total nutrients of milk. In many countries, by-products of the dairy industry are disposed of as sewage, which is a serious environmental hazard. To some extent, this problem has been solved by using waste of cheese production (whey) and buttermilk as a growth medium for lactobacilli [15,16]. However, the processes of clarifying whey and separating proteins involve extra expense.

Also, it was confirmed that sauerkraut juice used as a growth medium component was effective for the growth of bacteria *Lactobacillus plantarum S1*, *Lactobacillus plantarum ATCC 10241*, *Lactobacillus acidophilus LB45*, and *Lactobacillus casei LB10* [17]. There is evidence that using fermentative cabbage hydrolysate as the basis for growth media is effective, too [18].

Studies show that if sweet potato (*batata*) extracts are used as components of LAB culture media, the result is better than it is with a standard MRS medium. Besides carbohydrates, amino acids, vitamins, and minerals bacteria need for development, *batatas* contain antioxidants, triglycerides, linoleic and palmitic acids [19].

There is a lactobacilli culture medium that includes a cabbage decoction, glucose, and additional sources of nutrients: acid hydrolysate of slaughter animals' blood, autolysed yeast, and milk whey [20]. Another invention [21], which allows increasing the selectivity of a growth medium and modifying its production, is a medium for lactobacilli isolation that

includes cabbage decoction, glucose, agar, and other components. As additional nutrition sources providing its selectivity, the medium contains milk whey, autolysed yeast, and acetic acid.

Ukrainian researchers developed a growth medium that allowed *Lactobacillus plantarum* to accumulate cellular biomass and met its nutritional requirements. The research dealt with maize extract and fodder yeast lysate and hydrolysate and proved their effectiveness as the main nitrogen sources [22].

Studies confirmed that peptones obtained from cod's inner organs and included in growth media could perfectly maintain *Lactobacillus plantarum* biomass accumulation [23].

Search for new raw material sources for LAB culture media made it clear that fruit and vegetable processing industries should become an object of analysis, too. Waste of these industries includes 10–25% of tomato and apple pomace and of cabbage processing waste. Almost all of them are secondary resources of raw materials, since they contain valuable substances: vitamins, sugars, protein, microelements.

Ukraine is the world's fourth potato producer. In 2019, the total harvest of potatoes was 20.27 m tons; 10,000 tons of potato starch was manufactured [24]. Potato is one of the main crops grown in Ukraine. Its chemical composition depends on its variety, climate, soil, and other growing conditions, and ranges widely. As much as 5–6% of Ukrainian potatoes are processed into starch [24]. In our country, there are four big-scale enterprises that manufacture starch. They never stop expanding their capacity, because the existing manufacturing volumes are far below what Ukraine's home market needs – hence the increasing volumes of by-products of potato processing. Fig. 1 shows a process flow chart of obtaining starch from potatoes. According to the chart, the main by-products of the process are cellular fluid and juice-containing water.

In the technology of obtaining starch, centrifuging the pulp of crushed potatoes provides up to 45% of cellular juice containing 5.5–6.5% of dry matter [25]. When the pulp is washed, starch milk is obtained, which is then dewatered, and in the course of this process, juice-containing water is produced.

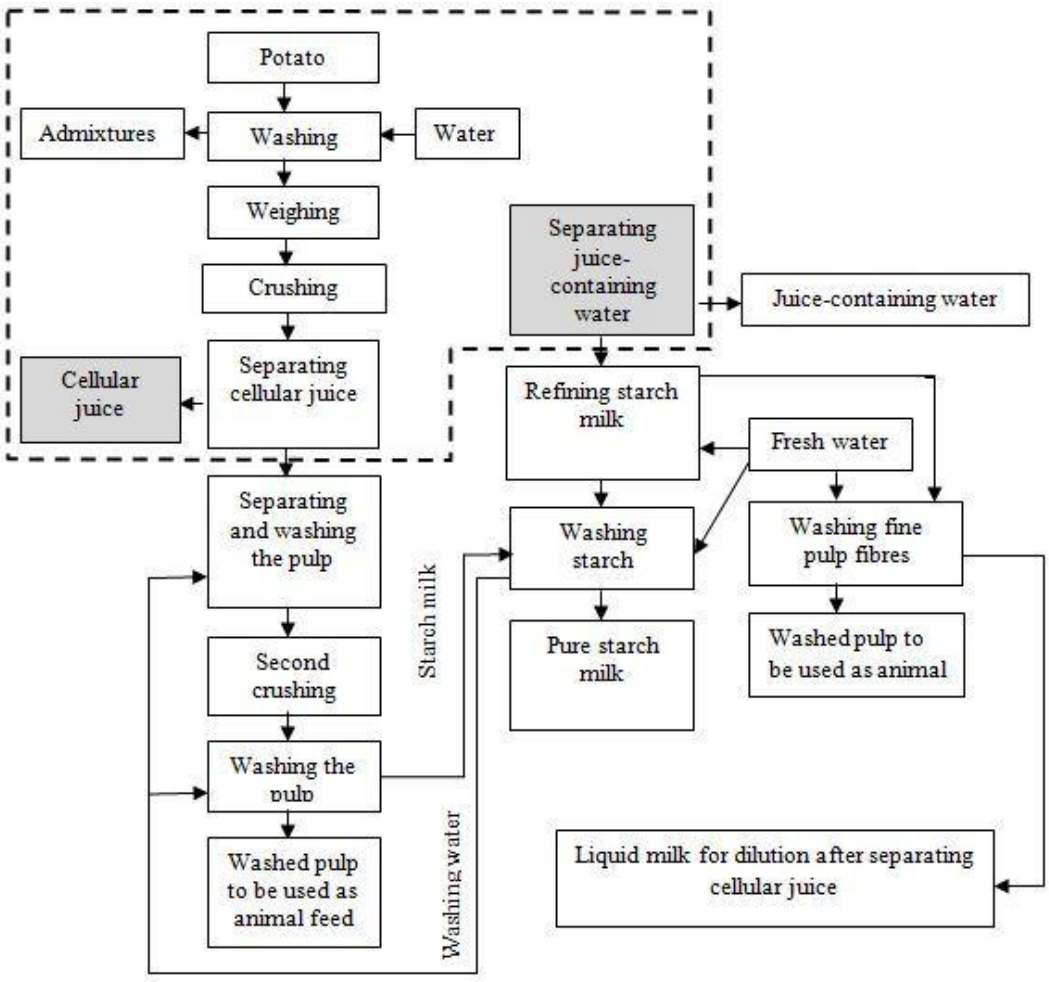

Fig. 1. Process flow chart of obtaining wet potato starch [24]

Its dry matter content can range 0.6–1%, depending on the technological process. Its chemical composition can vary, too, depending on the variety and the condition of the potatoes processed (whether they are fresh, or frost-damaged, etc.).

The chemical composition of potato processing products includes more than 27% of nitrogen compounds. Half of them are proteins that include all essential amino acids. The nitrogen-free substances are starch (no less than 5%), mineral substances (about 9–12%), sugars (up to 20%). The biochemical composition of products obtained from processing potatoes can be found in literature, and is presented in Table 2.

Table 2 – Biochemical composition of products obtained after processing potatoes into starch (%) [25]

Composition	Potato component		
	Whole tuber	Cellular juice	Juice-containing water
Starch	18.5	6.8	5.6
Cellulose	1.15	0.15	0.18
Soluble sugars	5.0	7.1	4.9
Nitrogen compounds	2.0	48.5	32.7
Minerals	1.0	5.8	16.3

Most enterprises do not use nutrients from juice-containing water and direct it to settling basins for purification. However, it can be quite costly for an enterprise to purify wastewater completely. The most commonly applied method of utilising juice-containing water is evaporating it till its moisture content is 10%, followed by using it to manufacture animal feed [25]. That is why utilisation of starch production waste is a promising alternative, because it makes the production process environment-friendly.

All the above proves that some growth medium ingredients can be replaced, partly or completely, with cheaper ones to grow *Lactobacillus*.

The **purpose** of this research is to study whether waste and by-products of potato starch production can be used as components of a culture medium for *Lactobacillus* bacteria.

To achieve this purpose, it was necessary to attain the following **objectives**:

– to compare the kinetics of LAB growth on different vegetable extracts;

– to study the specific features of culturing LAB on growth media based on by-products of processing potatoes into starch.

Research materials and methods

Materials under study: potato (the cultivars *Synyo-oka* and *Bellarosa*), cabbage (the cultivars *Caporal F1* and *Coronet*), tomatoes (the cultivars *Rio Grande* and *Bobcat F1*), vegetable extracts. For the experiments, 4 variants of test samples were used to culture LAB in. The extracts were prepared as follows.

Sample 1. 500 g of fresh white cabbage was ground in a laboratory blender till the particle size was 2–5 mm. Then, it was covered with tap water (in the ratio 1:2), heated for 8–10 min at 100°C, cooled down to 35–40°C, and filtered.

Sample 2. Juice-containing water from the potatoes was obtained as follows. 500 g of the ground raw material (particle size 1–2 mm) was covered with 1 litre of tap water, heated for 8–10 min at 100°C, cooled down to 35–40°C, and filtered.

Sample 3. The tomatoes were ground in a laboratory blender till the size of the particles was 1–3 mm 500 g of the pulp was prepared in the same way as Samples 1 and 2.

Sample 4. Potato juice was obtained as follows. The potatoes were washed in running water, peeled, ground in a blender (particle size 1–2 mm), and the pulp obtained was crushed on a laboratory press.

Before inoculation, all the samples were sterilised for 15–20 min at 100°C.

The research involved using gram-positive rod-shaped anaerobic nonsporulating lactic acid bacteria of the strain *Lactobacillus plantarum 8R-A3*. A bottle with the freeze-dried LAB strain was opened under aseptic conditions, and 5ml of a medium was introduced. The contents of the bottle was then dissolved and reinoculated into flasks with the media under study. The lactobacilli were cultured by deep inoculation in a thermostat (TS-80M-2) at 37°C for 24 hours. The biomass accumulation was regularly monitored. The proportion of the microorganisms introduced into the growth media was 10^5CFU/cm^3.

The media were selected and optimised according to the following criteria: pH of a media, thermal conditions, quantity and weight of microbial cells after incubation. The pH of the growth media was measured by standard methods using a pH meter. The thermal condition of the culture production was determined by the common methods.

To measure the bacterial growth and biomass accumulation, traditional methods were used, the same ones as those normally used in a production environment: measuring by the weight method and by the bacterial optical density [26]. When using the weight method, the culture fluid samples were selected, centrifuged, washed with water on Schott glass filters, and dried at 110°C for 8 hours in a drying cabinet. The optical density of the cultures was measured with a photocolourimeter KFK-2 at λ=540 nm and with a 1.0 cm thick cuvette.

Lactobacilli were grown in the biotechnological laboratory at the Bio-Engineering and Water Department, Odessa National Academy of Food Technologies.

Results of the research and their discussion

The main by-products in potato starch production are cellular juice and juice-containing water [25]. That is why these secondary raw materials of starch production are the ones we have suggested using as a component of a lactobacilli growth medium.

Table 3 – Correlation of the number of cells of lactic acid bacteria biomass with the wet and dry weight of bacteria (cultured on classical MRS agar media)

Number of cells per 1 cm^3 of the culture fluid	Wet weight of LAB, mg/l	Number of CFU in 1mg of LAB wet weight	Dry weight of LAB, mg/l	Number of CFU in 1mg of LAB dry weight
$3 \cdot 10^8$	5970	$5.2 \cdot 10^7$	335	$9.5 \cdot 10^8$
$2 \cdot 10^8$	4980	$4.0 \cdot 10^7$	275	$7.2 \cdot 10^8$

To compare the growth kinetics of LAB, traditional components of synthetic growth media (tomato and cabbage extracts) were used as substrates [17-21].

To study the growth and activity of lactic acid bacteria, a number of methods are used: direct count, volumetric, by weight, by the optical density (turbidity), as well as determining the culture fluid viscosity. It is known what ratio is between the turbidity of bacterial suspensions and the dry weight of bacteria grown on classic media [27]. To achieve the biomass concentration 30g/l, one needs to spend 60g of sugars to feed lactic acid bacteria. Thus, the above-mentioned sugar content in the cellular juice and juice-containing water allows achieving the cell concentration 20–30g/l. Table 3 presents the correlation of the number of LAB cells per 1 cm^3 with dry and wet weight (mg/l).

Fig. 2 shows the result of *Lactobacillus plantarum* biomass accumulation after culturing for 96 hours at 37°C on media of different composition. The samples suggested (2 and 4) have shown that LAB biomass accumulates far better than it does in the samples using the traditional components of synthetic growth media for lactic acid bacteria.

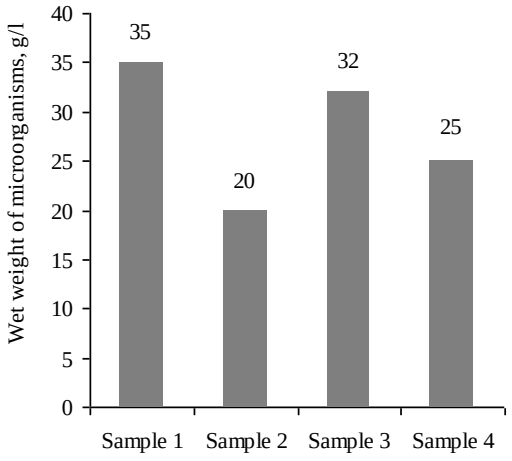

Fig. 2. Comparison of accumulation of *Lactobacillus plantarum* biomass

To provide proper conditions allowing microorganisms to consume nutrients, growth media must have the optimum concentration of hydrogen ions (pH). The research considers how the pH of the growth media samples (extracts) changes in the course of LAB culturing. The findings are given in Table 4. They indicate acid accumulation in the test samples.

The findings indicate the accumulation of lactic acid in the samples under study. It has been established that the substrate based on cellular juice (Sample 4) is highly fermentable, as compared with the tomato and cabbage extracts. The changes in the pH in Samples 2 (juice-containng water) and 4 (potato juice) are due to different compositions of the substrates.

Table 4 – Changes in the pH of the growth media samples during culturing

Sample number	Duration of culturing, τ (hours) at t=37°C, pH=5.5				
	0	24	48	72	96
Sample 1	5.40	4.33	4.20	3.60	3.40
Sample 2	6.50	4.36	4.31	3.94	3.90
Sample 3	6.30	4.00	4.00	3.80	3.70
Sample 4	5.50	4.19	4.00	3.70	3.50

The parameter indicating the growth of the bacterial biomass during thermostatting is the viscosity of the culture fluid [27]. This parameter has been determined in the samples studied. Changes in it are evidence of LAB biomass accumulation (Fig. 3).

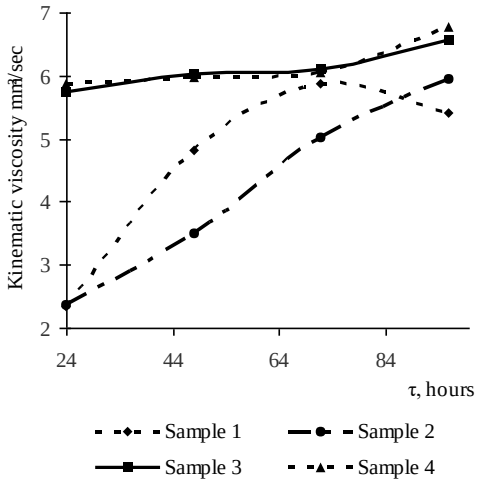

Fig. 3. Changes in the culture fluid viscosity during thermostatting

The culture liquids based on the tomato extract and on the potato juice have almost the same kinematic viscosity values. The lowest viscosity is that of the sample based on the juice-containing water from potatoes. Since search for cheap, high production volume substrates to grow LAB on is a promising approach, further studies focused on the samples with the potato juice and juice-containing water, which are by-products of starch production.

The lactic acid bacteria growth on potato juice has been analysed by the weight method. The data obtained indicate that, compared to the traditional growth media components, potato juice is effective, too. This is clear from the increase of the biomass of *Lactobacillus plantarum* (Fig. 4), which was $3 \cdot 10^8$ CFU/cm^3 after 96 hours of culturing in a thermostat.

Cellular juice and juice-containing water differ in their dry matter content, because at industrial enterprises, pulp is washed with water many times. Correspondingly, the bacteria biomass growth is different. However, this did not have a considerable effect on the wet weight accumulation in the lactic acid bacteria *Lactobacillus plantarum*.

The kinetics of lactobacilli growth was determined by measuring the optical density of the solution, and the findings are shown in Fig. 5.

As early as on the second day, the biomass increase slowed down. After 3.5 days, there was practically no change in the kinetics of LAB growth. After 96 hours of culturing, the final wet weight of *Lactobacillus plantarum* was 20 g/l (on juice-containing water) and 25 g/l (on potato juice).

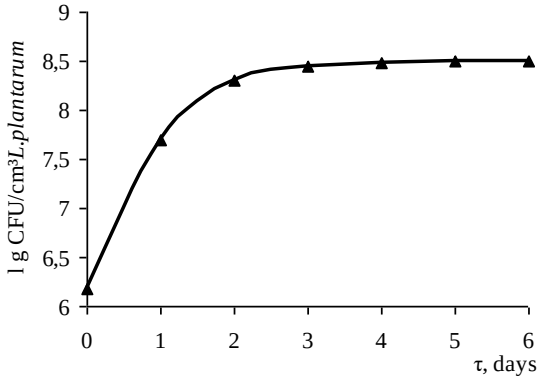

Fig. 4. Dependence of biomass accumulation in *Lactobacillus plantarum* on the duration of culturing

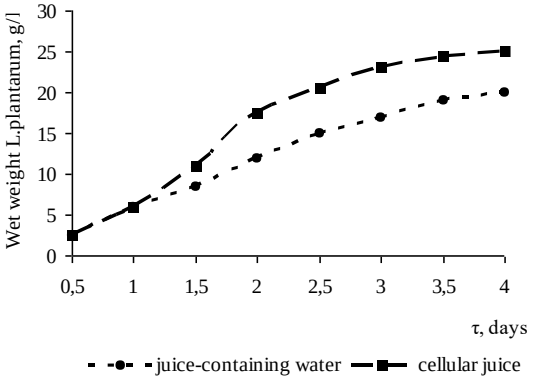

Fig. 5. Kinetics of the growth of *Lactobacillus plantarum* on cellular juice and on juice-containing water (pH=6.5, t=37°C)

Conclusion

The paper analyses the prospects of using food production waste as cheap components of growth media to culture lactic acid bacteria on. Based on the literature analysed and on the theoretical material summed up, the by-products of potato processing (cellular juice and juice-containing water) were selected to be used as components of culture media for bacteria of the genus *Lactobacillus.* The kinetics of lactic acid bacteria growth on different vegetable extracts has been compared. It has been proved that quite a lot of juice-containing water to be utilised by a starch-processing plant, can also be effectively used in creating culture media for *Lactobacillus plantarum*. The accumulation of wet biomass of *Lactobacillus plantarum* on juice-containing water and on potato juice after 96 hours of culturing, was, respectively, 20g/l and 25g/l, with the number of bacteria $3 \cdot 10^8$CFU/cm^3. The research results prove that using by-products of potato starch production can be a good alternative when developing the optimal composition of culture media for lactic acid bacteria of the genus *Lactobacillus.*

References:

1. Splittstoesser DF, Vanderzant C. Compendium of Methods for the Microbiological Examination of Foods (4rd ed.). Washington (D.C.): American public health association; 2001.
2. Walstra P, Wouters JTM, Geurts TJ. Dairy Science and Technology. London, New York; 2006.
3. Sovremennyie problemyi nauki i obrazovaniya. Bespomestnyih K. Izuchenie vliyaniya sostava pitatelnoy sredyi na izmenenie biohimicheskih i morfologicheskih svoystv shtammov laktobatsill. [Internet].2014 Dek 25 [cited 2020 May 20] Available from: http://www.science-education.ru/ru/article/view?id=16600.
4. Savijoki K, Ingmer H, Varmanen P. Proteolytic systems of lactic acid bacteria. Applied Microbiology and Biotechnology. 2006;71:394-406. https://doi.org/10.1007/s00253-006-0427-1.
5. Wegkamp A, Teusink B, De Vos WM, Smid EJ. Development of a minimal growth medium for Lactobacillus plantarum. Applied Microbiology. 2010;50(1):57-64. https://doi.org/10.1111/j.1472-765X.2009.02752.x.
6. Ewe J, Liong M. Viability and growth characteristics of Lactobacillus in soymilk supplemented with B-vitamins. International Journal of Food Sciences and Nutrition. 2010;61(1):87-107. https://doi.org/10.3109/09637480903334163.
7. Hebert EM, Raya RR, Savoy de Giori G. Evaluation of Minimal Nutritional Requirements of Lactic Acid Bacteria Used in Functional Foods. Methods in Biotechnology. [Internet]. 2004. [cited 2020 July 30]. Available from: https://www.researchgate.net/profile/Rakesh_S_PILLAI/publication/256842590_Environmental-Microbiology-Methods-and-Protocols/links/00b7d523edc4be687e000000/Environmental-Microbiology-Methods-and-Protocols.pdf.
8. Calderon M, Loiseau G, Guyot JP. Nutritional requirements and simplified cultivation medium to study growth and energetics of a sourdough lactic acid bacterium Lactobacillus fermentum Ogi E1 during heterolactic fermentation of starch. Applied Microbiology. 2001;90(4):508-516. https://doi.org/10.1046/j.1365-2672.2001.01272.x.
9. Vazquez JA, Gonzalez MP, Murado MA. Peptones from autohy-drolysed fish viscera for nisin and pediocin production. Journal of Biotechnology. 2004;112(3):299-311. https://doi.org/10.1016/j.jbiotec.2004.04.011.

10. HiMedia Laboratories Pvt. Ltd. Agar MRS (Man, Rogoza, Sharp) [Internet]. Moskov: HiMedia Laboratories Pvt. Ltd. Available from: http://www.himedialabs.ru/
11. Djeghri-Hocine B, Boukhemis M, Zidoune MN, Amrane A. Evaluation of de-lipidated egg yolk and yeast autolysate as growth supplements for lactic acid bacteria culture. International Journal of Dairy Technology. 2007;60(4):292-296. https://doi.org/10.1111/j.1471-0307.2007.00351.x.
12. Aguirre L, Garro MS, Savoy de Giori G. Enzymatic hydrolysis of soybean protein using lactic acid bacteria. Food Chemistry. 2008;111(4):976-982. http://dx.doi.org/10.1016/j.foodchem.2008.05.018.
13. Altaf MD, Naveena BJ, Reddy G. Use of inexpensive nitrogen sources and starch for l(+) lactic acid production in anaerobic sub-merged fermentation. Bioresource Technology. 2007;98(3):498-503. https://doi.org/10.1016/j.biortech.2006.02.013.
14. Hayek SA, Gyawali R, Aljaloud SO, Krastanov A. Cultivation media for lactic acid bacteria used in dairy products. The Journal of dairy research. 2019;86(4):490-502. https://doi.org/10.1017/S002202991900075X.
15. Bovo F, Franco LT, Rosim RE, Fernandes de Oliveira CA. Ability of a Lactobacillus rhamnosus strain cultured in milk whey based medium to bind aflatoxin B1. Food Science and Technology. 2014;34(3):566-570. https://doi.org/10.1590/1678-457x.6373.
16. Soenarno MS, Sumantri C, Taufik E, Nuraida L, Arief II. Lactobacillus plantarum IIA-1A5 fermentation patterns by using whey, buttermilk and whey enriched by skimmed milk as growth media. Pakistan Journal of Nutrition. 2019;18(3):288-295. http://doi.org/10.3923/pjn.2019.288.295.
17. Champagne CP, Savard T, Barrette J. Production of lactic acid bacteria on spent cabbage juice. Journal of food, agriculture & environment. 2009;7(2):82-87. https://doi.org/10.1234/4.2009.1543.
18. Timchenko LD, Penkova NI, Katutina LS. Sravnitelnyiy analiz traditsionnyih pitatelnyih sred i novaya kapustnaya sreda dlya kultivirovaniya laktobakteriy. Vestnik MGOU. Seriya Estestvennyie nauki. 2010;2:51-55.
19. Hayek SA, Aboghasem Shahbazi A, Awaisheh SS, Shah NP, Ibrahim SA. Sweet potatoes as a basic component in developing a medium for the cultivation of lactobacilli. Bioscience, Biotechnology and Bio-chemistry. 2013;77(11):2248-2254. https://doi.org/10.1271/bbb.130508.
20. Terehov VI, Nasonova IN, Serdyuchenko IV, Burdyug AI, Mishel Mufid Abaui, Bukurova VA, izobretateli; Federal. gos. byud. obraz. uchrezhd. vyssh. prof. obraz. Kubanskij gos. agrar. un-t., sobstvennik. Pitatelnaya sreda dlya kultivirovaniya laktobakteriy. Pat. Russia № RU2551960C1. 2015 June 10.
21. Terekhov VI, Arushanyan AY, Serdyuchenko IV, Glushchenko SG, izobretateli; Federal. gos. byud. obraz. uchrezhd. vyssh. prof. obraz. Kubanskij gos. agrar. un-t, sobstvennik. Kulturalnaya sreda dlya vyideleniya laktobatsill. Pat. Russia № RU2510416C1. 2014 March 27.
22. Sorokulova IB, Khilko TV, Osadchaia AI. Development of nutrient medium for the cultivation of Lactobacillus plantarum 8R-A3. Mikrobiolohichnyi Zhurnal. 2003;65(3):39-45.
23. Horn SJ, Aspmo SI, Eijsink VG. Growth of Lactobacillus plantarum in media containing hydrolysates of fish viscera. Journal of Applied Microbiology. 2005;99(5):1082-1089. https://doi.org/10.1111/j.1365-2672.2005.02702.x.
24. Kulakova O. Starch direction: where Ukraine is heading. [Internet]. Kiyiv: AgroPortal; 2015 [updated 2020 June 17., cited 2020 July 30]. Available from: http://agroportal.ua/views/blogs/krakhmalnoe-napravlenie-kuda-dvizhetsya-ukraina/#
25. Trehubov NN, redactor. Tekhnolohiya krakhmalov i krakhmaloproduktov. Moskva: Lehkaya i pishchevaya promyshlennost; 1981.
26. Shlegel G. Obshhaya mikrobiologiya: per. s nem. Moskva: Mir; 1987.
27. Ribero-Gajon Zh, Pejno E, Ribero-Gajon P, Syudro P. Teoriya i praktika vinodeliya. Tom 2: per. s francz. Moskva: Pishhevaya promyshlennost; 1979.

ПЕРСПЕКТИВИ ВИКОРИСТАННЯ ПОБІЧНИХ ПРОДУКТІВ ВИРОБНИЦТВА КАРТОПЛЯНОГО КРОХМАЛЮ ЯК КОМПОНЕНТІВ ЖИВИЛЬНИХ СЕРЕДОВИЩ ДЛЯ КУЛЬТИВУВАННЯ МОЛОЧНОКИСЛИХ БАКТЕРІЙ

А.Т. Безусов, докт. техн. наук, професор, *E-mail*: anatoliy.bezusov2@gmail.com
Т.М. Афанасьєва, кандидат технічних наук, доцент, E-*mail*: tana79.a@gmail.com
Н.В. Доценко, кандидат технічних наук, доцент, E-*mail*: n-dotsenko@ukr.net
Г.І. Палвашова, кандидат технічних наук, доцент, E-*mail*: palvashova_ai@ukr.net
Кафедра біоінженерії і води
Одеська національна академія харчових технологій, вул. Канатна, 112, м. Одеса, Україна, 65039

Анотація. Статтю присвячено пошуку дешевих альтернативних компонентів для субстратів живильних середовищ при культивуванні молочнокислих бактерій. Широке застосування лактобактерій в харчовій промисловості потребує збільшення об'ємів їх виробництва. Підприємства, які вирощують молочнокислі бактерії, а також виробляють пробіотичні препарати зацікавлені в використанні нетрадиційних компонентів живильних середовищ. Складність створення дешевих живильних середовищ обумовлена тим, що лактобактерії дуже вимогливі до вмісту окремих компонентів. Традиційні середовища для культивування можуть містити до 40 компонентів (пептони, дріжджові та овочеві екстракти, вітаміни, тощо). Доведено можливість використання відходів та побічних продуктів виробництва картопляного крохмалю як компонента субстратів для культивування лактобактерій. У роботі використовували штам *Lactobacillus plantarum 8R-A3*. Проведено аналіз кінетики росту молочнокислих бактерій на різних овочевих екстрактах. Екстракти томатів та капусти використовували як традиційні компоненти синтетичних середовищ для порівняння. Показано, що на субстраті з побічних продуктів виробництва картопляного крохмалю, накопичення біомаси *Lactobacillus plantarum* при культивуванні протягом 96 годин за температури 37°С складає 20 г/л (на соковій воді) та 25 г/л (на картопляному соці). Ефективність використання побічних продуктів виробництва картопляного крохмалю у якості компонентів живильного середовища підтверджено визначенням кінематичної в'язкості культуральної рідини, яка склала для картопляного соку 6,77мм^2/с після термостатування, що дещо вище за в'язкість томатного та капустяного екстрактів. Оскільки крохмалепатокове виробництво за своїми об'ємами зростає кожного року, відповідно зростає і кількість побічних продуктів переробки картоплі. Тому, надалі було запропоновано картопляний сік та сокову воду як альтернативу традиційним субстратам. Підтверджено, що вторинні продукти переробки картоплі забезпечують приріст біомаси молочнокислих бактерій в достатній кількості. На четверту добу кількість бактерій склала $3 \cdot 10^8$ КУО/см^3.

Обґрунтовано доцільність використання вторинних продуктів переробки картоплі як субстратів живильних середовищ при культивуванні молочнокислих бактерій роду *Lactobacillus plantarum.*

Ключові слова: живильні середовища, культивування, молочнокислі бактерії, *Lactobacillus plantarum*, відходи харчових виробництв, виробництво крохмалю.

Список літератури

1. Splittstoesser Don F., Vanderzant Carl. Compendium of Methods for the Microbiological Examination of Foods (4rd ed.). Washington (D.C.): American public health association, 2001. 1219 p.
2. Walstra P., Wouters J.T.M., Geurts T.J. Dairy Science and Technology. CRC Taylor & Francis Group: Boca Raton, London, New York, 2006. 782 p.
3. Беспоместных К. Изучение влияния состава питательной среды на изменение биохимических и морфологических свойств штаммов лактобацилл // Современные проблемы науки и образования*:* веб-сайт. URL: http://www.science-education.ru/ru/article/view?id=16600 (дата звернення: 29.07.2020).
4. Savijoki K., Ingmer H., Varmanen P. Proteolytic systems of lactic acid bacteria // Applied Microbiology and Biotechnology. 2006. Vol. 71. P. 394-406. https://doi.org/10.1007/s00253-006-0427-1.
5. Development of a minimal growth medium for Lactobacillus plantarum / Wegkamp A. et al. // Applied Microbiology. 2010. Vol. 50, No. 1. P. 57-64. https://doi.org/10.1111/j.1472-765X.2009.02752.x.
6. Ewe J., Liong M. Viability and growth characteristics of Lactobacillus in soymilk supplemented with B-vitamins // International Journal of Food Sciences and Nutrition. 2010. Vol. 61, No. 1. P. 87-107. https://doi.org/10.3109/09637480903334163.
7. Elvira M. Hébert, Raúl R. Raya, Graciela Savoy de Giori. Evaluation of Minimal Nutritional Requirements of Lactic Acid Bacteria Used in Functional Foods // Methods in Biotechnology. 2004. No. 14. P. 139-150. URL: https://www.researchgate.net/profile/Rakesh_S_PILLAI/publication/256842590_Environmental-Microbiology-Methods-and-Protocols/links/00b7d523edc4be687e000000/Environmental-Microbiology-Methods-and-Protocols.pdf (viewed 30.07.2020).
8. Calderon M., Loiseau G., Guyot J.P. Nutritional requirements and simplified cultivation medium to study growth and energetics of a sourdough lactic acid bacterium Lactobacillus fermentum Ogi E1 during heterolactic fermentation of starch // Applied Microbiology. 2001. Vol. 90, No. 4. P. 508-516. https://doi.org/10.1046/j.1365-2672.2001.01272.x.
9. Vazquez J.A., Gonzalez M.P., Murado M.A. Peptones from autohy-drolysed fish viscera for nisin and pediocin production // Journal of Biotechnology. 2004. Vol. 112, No. 3. P. 299-311. https://doi.org/10.1016/j.jbiotec.2004.04.011.
10. Агар MPC (Ман, Рогоза, Шарп). *HiMedia Laboratories*: веб-сайт. URL: http://www.himedialabs.ru/. (дата звернення: 29.07.2020).
11. Evaluation of de-lipidated egg yolk and yeast autolysate as growth supplements for lactic acid bacteria culture / Djeghri-Hocine B. et al. // International Journal of Dairy Technology. 2007. Vol. 60, No. 4. P. 292–296. https://doi.org/10.1111/j.1471-0307.2007.00351.x.
12. Aguirre L., Garro M.S., Savoy de Giori G. Enzymatic hydrolysis of soybean protein using lactic acid bacteria // Food Chemistry. 2008. Vol. 111, No. 4. P. 976-982. http://doi.org/10.1016/j.foodchem.2008.05.018.
13. Altaf M.D., Naveena B.J., Reddy G. Use of inexpensive nitrogen sources and starch for l(+) lactic acid production in anaerobic submerged fermentation // Bioresource Technology. 2007. Vol. 98, No. 3. P. 498-503. https://doi.org/10.1016/j.biortech.2006.02.013.
14. Cultivation media for lactic acid bacteria used in dairy products / Hayek S.A. et al. // The Journal of dairy research. 2019. Vol. 86, No. 4. P. 490-502. https://doi.org/10.1017/S002202991900075X.
15. Ability of a Lactobacillus rhamnosus strain cultured in milk whey based medium to bind aflatoxin B1 / Bovo F. et al. // Food Science and Technology. 2014. Vol. 34, No. 3. P. 566-570. https://doi.org/10.1590/1678-457x.6373.
16. Lactobacillus plantarum IIA-1A5 fermentation patterns by using whey, buttermilk and whey enriched by skimmed milk as growth media / Mohammad Sriduresta Soenarno et al. // Pakistan Journal of Nutrition. 2019. Vol. 18, No. 3. P. 288-295. http://doi.org/10.3923/pjn.2019.288.295.
17. Champagne C.P., Savard T., Barrette J. Production of lactic acid bacteria on spent cabbage juice // Journal of food, agriculture & environment. 2009. Vol. 7, No. 2. P. 82-87. https://doi.org/10.1234/4.2009.1543.
18. Тимченко Л.Д., Пенькова Н.И., Катутина Л.С. Сравнительный анализ традиционных питательных сред и новая капустная среда для культивирования лактобактерий // Вестник МГОУ. Серия Естественные науки. 2010. № 2. С. 51-55.
19. Sweet potatoes as a basic component in developing a medium for the cultivation of lactobacilli / Hayek S.A. et al. // Bioscience, Biotechnology and Bio-chemistry. 2013. Vol. 77, No. 11. P. 2248-2254. https://doi.org/10.1271/bbb.130508.
20. Питательная среда для культивирования лактобактерий: пат. RU2551960C1 Россия: МПК C12N 1/20 / Терехов В.И., Насонова И.Н., Сердюченко И.В., Бурдюг А.И., Мишель Муфид Абауи, Букурова В.А.; собственник Федерал. гос. бюд. образ. учрежд. высш. проф. образ. Кубанский гос. аграр. ун-т. № 2014112659/10; заявл. 01.04.2014; опубл. 10.06.2015, Бюл. № 16.
21. Питательная среда для выделения лактобактерий: пат. RU2510416C1 Россия: МПК C12N 1/20 / Терехов В.И., Арушанян А.Я., Сердюченко И.В., Глущенко С.Г.; собственник Федерал. гос. бюд. образ. учрежд. высш. проф. образ. Кубанский гос. аграр. ун-т. № 2012154888/10; заявл. 18.12.2012; опубл. 27.03.2014, Бюл. № 9.
22. Sorokulova I.B., Khil'ko T.V., Osadchaia A.I. Development of nutrient medium for the cultivation of Lactobacillus plantarum 8R-A3 // Mikrobiolohichnyi Zhurnal. 2003. Vol. 65, No. 3. P. 39-45.
23. Horn S.J., Aspmo S.I., Eijsink V.G. Growth of Lactobacillus plantarum in media containing hydrolysates of fish viscera // Journal of Applied Microbiology. 2005. Vol. 99, No. 5. P. 1082-1089. https://doi.org/10.1111/j.1365-2672.2005.02702.x.
24. Кулакова О. Крахмальное направление: куда движется Украина. *Agro Portal*: веб-сайт. URL: http://agroportal.ua/views/blogs/krakhmalnoe-napravlenie-kuda-dvizhetsya-ukraina/#. (дата звернення 30.07.2020).
25. Технология крахмалов и крахмалопродуктов / Трегубов Н.Н. и др.; под ред. Н.Н. Трегубова. Москва: Легкая и пищевая пром-ть, 1981. 472 с.
26. Шлегель Г. Общая микробиология: пер. с нем. Москва: Мир, 1987. 567 с.
27. Риберо-Гайон Ж., Пейно Э., Риберо-Гайон П., Сюдро П. Теория и практика виноделия. Том 2: пер. с франц. Москва: Пищевая пром-сть, 1979. 352 с.