Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2020, 10(3), 83-87, doi: 10.15421/2020_137

ORIGINAL ARTICLE

Enhanced cultivation technology for lacto- and bifidobacteria

A.P. Paliy[1*], S.A. Gujvinska[1], K.O. Rodionova[2], N.V. Alekseeva[3], O.V. Ponomarenko[4], M.S. Alrawashdeh[5], T.A. Yeletskaya[6], G.V. Ponomarenko[4], V.Yu. Kushnir[7], A.P. Palii[8]

[1]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, 83 Pushkinska St, Kharkiv, 61023, Ukraine
[2]Luhansk National Agrarian University, 68, Slobojanska Str., Starobilsk, 92703, Ukraine
[3]Dnipro State Agrarian and Economic University, 25 Sergii Yefremov St, Dnipro, 49600, Ukraine
[4]Kharkiv State Zooveterinary Academy, 1 Akademicheskaya St, urban settlement Malaya Danilivka, Dergachivsky district, Kharkov region, 62341, Ukraine
[5]University Mutah, P.O Box. 7, AL Karak, 61710, Jordan
[6]Institute of Animals Science of the National Academy of Agricultural Sciences, 1A Givotnovodov St, Kharkiv, 61026, Ukraine
[7]Odessa State Agrarian University, 13 Panteleimonovskaya St, Odessa, 65012, Ukraine
[8]Kharkiv National Technical University of Agriculture named after Petro Vasylenko, 44 Alchevskih St, Kharkiv, 61002, Ukraine

***E-mail:** paliy.dok@gmail.com*

Received: 04.06.2020. Accepted: 12.07.2020

The aim of this work was to improve a cultivation technology of lactobacteria and bifidobacteria based on the optimal growth substrate, which ensures a preservation of basic biological properties of microorganisms. Seven variants of experimental nutrient media were prepared for cultivation of lactic acid bacteria. The growth properties of experimental nutrient media were studied using following strains: *Lactobacillus plantarum 7*, *L. casei 27*, *L. plantarum 7-317*, *Bifidobacterium adolescentis 17* and *B. adolescentis 17-316*. The number of living microbial cells was determined by serial dilution methods. The composition of nutrient media for cultivation of lactobacteria, bifidobacteria and technological parameters of their cultivation were determined by the temperature of 37 ± 0.5°C. Acidity of a nutrient medium has pH value ranges from 6.5 to 7.5. A nutrient medium for cultivation of lactobacteria is proposed. Composition of new nutrient medium: lactose – 10.0-15.0%, sucrose – 5.0-10.0%, cystine – 0.01%, skim milk – up to 100.0%. It was established that the optimal nutrient medium for bifidobacteria accumulation composed of the following components (ratio of components, mass %): peptone – 7.0 ± 0.01, yeast autolysate – 4.0 ± 0.01, glucose – 1,5 ± 0.02, sucrose – 1.5 ± 0.01, cystine – 0.03 ± 0.01, ammonium citrate – 0.4 ± 0.03, potassium phosphate monosubstituted – 0.4 ± 0.02, MgSO4 – 0.04 ± 0.002, MnSO4 – 0.007 ± 0.002, sodium phosphate disubstituted – 0.4 ± 0.02, microbiological agar – 2.5 ± 0.04, sodium citrate – 0.8 ± 0.05, distilled water – up to 100%.

Key words: Cultivation technology; Animals; Biological studies

Introduction

Livestock farms implement drugs with probiotic cultures in schemes of prevention and treatment of animals within connection with the increasing needs of population in high-quality and safe food products of animal origin. Today, lactic acid cultures are essential components for the production of probiotics, as their positive effects have been repeatedly proven (Bermudez-Brito et al., 2012; Yirga, 2015). Probiotics have a positive effect on intestinal flora of animals (Markowiak & Śliżewska, 2018). It also reduces a risk of gastrointestinal disease in animals and increases their productivity (Chaucheyras-Durand & Durand, 2010). This statement refers to antibiotics, which are used for therapeutic purposes. An antibiotic resistance of many microorganisms is found to antibiotics (Hossain et al., 2017; Hadzevych et al., 2019). Biotechnological advancement has made it possible to create a complex probiotic additives consisting of several different strains and types of microorganisms (Zhu et al., 2018). This trend becomes very promising, since these strains complement each other and exhibit a more effective synergistic, prophylactic and metabolic effect in comparison with monotherapy (Gadde et al., 2017). Current requirements of the European regulatory legislation in the field of probiotics provide the need for comprehensive studies of biological activity of individual probiotic cultures and their combinations. These studies has to be implemented for creating probiotic additives based on monocultures of lactobacteria and bifidobacteria or their various combinations (Alayande et al., 2020). Study of biological properties of lactobacteria and bifidobacteria, as well as other microorganisms, requires an appropriate skill in long-term storage and cultivation of cultures (Gujvinska et al., 2018). This is necessary both for a maintaining collections of lactic acid bacteria in a highly active state and for a manufacture and storage of probiotic drugs (Herawati et al., 2020). Conducted biological studies have allowed to isolate a significant spectrum of bacteria, such as: *Lactobacillus* spp., *Bifidobacterium* spp. and *Lactococcus* spp. These isolates can be used in the development of probiotic drugs for farm animals (Chiang et al., 2015; Gujvinska & Paliy, 2018b; Paliy et al., 2020). Great attention in the biotechnological process of creating therapeutic and prophylactic probiotics is given to two main factors. These factors are an achievement of a maximum level of biomass yield of viable bacterial cells and a biologically active substances synthesized by them (Parvez et al., 2006). It is important to consider adaptability under production conditions and stability of strains during cultivation, as well as taking into

account its probiotic properties preservation during selection (Ozen & Dinleyici, 2015). These indicators determine the productivity, competitiveness and profitability of technological process (Gujvinska & Paliy, 2018a).

Lactic acid bacteria and bifidobacteria are introduced into the composition of probiotics, dietary supplements and functional foods. They are widely used in the prevention and treatment of many animal dysfunctions (Patel et al., 2012; Celiberto et al., 2017). In recent years, attention is paid to the use of lactic acid bacteria growth promoters for increase the effectiveness of probiotics (Uzunova-Doneva & Donev, 2005). Separate data are presented regarding the effect of lactulose, inulin and aerosil on the growth of lactobacteria. However, there is no evidence about an effect of phytocompositions on the biological properties of lactic acid bacteria. A study of these issues is relevant because it reveals an another page of biotechnology of an important group of microorganisms known as lactobacteria and bifidobacteria (Ben Salah et al., 2012; Chiu et al., 2014; Wang et al., 2015).

These circumstances call for finding a new composition of nutrient medium based on components from domestic raw materials. This will reduce a cost of the nutrient medium and increase its elective properties, accelerate the growth of lactobacilli and bifidobacteria in general, and the accumulation of their bacterial mass in particular.

The aim of this work was to improve a cultivation technology of lactobacteria and bifidobacteria based on the optimal growth substrate.

Materials and Methods

A work for improvement of cultivation technology of lactobacteria and bifidobacteria based on optimal growth substrate was performed in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine". Seven variants of experimental nutrient media were prepared for cultivation of lactobacteria and bifidobacteria for the research study. Lactose, sucrose, cystine, and skim milk were used to design the medium for lactobacteria. Peptone, yeast autolysate, glucose, sucrose, cystine, ammonium citrate, potassium phosphate monosubstituted, magnesium sulfate, manganese sulfate, sodium phosphate disubstituted, sodium citrate, microbiological agar, distilled water were used in a medium for cultivation of bifidobacteria. The MRS medium was used as a control of growth properties of probiotic cultures (Nwamaioha & Ibrahim, 2018).

Selection and optimization of nutrient media were carried out according to the following criteria: pH of a medium, temperature, a number of microbial cells after incubation (Yang et al., 2018). Cultures of lactobacteria (*Lactobacillus plantarum 7*, *L. casei 27*, *L. plantarum 7-317*) and bifidobacteria (*Bifidobacterium adolescentis 17, B. adolescentis 17-316*) have been a subject of the research study. The abovementioned cultures are isolated, selected and stored in the Laboratory of Veterinary Sanitation and Parasitology of the NSC "IECVM". The number of live microbial cells assessed the growth properties of bifidobacteria. The number of live microbial cells was determined by the method of serial dilutions of a resulting suspension in saline. Subsequently, bacterial cultures were inoculated on nutrient media in a volume 0.1 cm^3 of the 10^{-6} dilution (Süle et al., 2014; Senz et al., 2015). The optimal conditions (pH, temperature) of cultivation of lactic acid bacteria were determined (Kneifel, 2000).

All studies were performed in triplicate. Statistical processing of the results was carried out using traditional methods of variation statistics using the "Microsoft Excel" and "Statistica 10" programs. A significant difference between obtained results was considered at p-value < 0.05.

Results and Discussion

A nutrient media with various sources of carbon and nitrogen were used for cultivation microorganisms. There are MRS control medium (glucose is a carbon source; enzyme peptone, meat broth, ammonium citrate are nitrogen sources) and seven experimental nutrient media for cultivation of lactobacilli (n=4) and bifidobacteria (n=3). These nutrient media differed by the type and concentration of carbon and nitrogen sources and mineral components.

A comparative analysis of well-known nutrient media for the cultivation of lactobacilli and bifidobacteria shows that successful cultivation depends on a quality and composition of a medium due to a type of protein base as well as a specificity of growth components (Sánchez et al., 2019). Thus, many researchers used glucose, lactose, and sucrose as carbohydrate sources to prepare nutrient media. In addition to this, various microelements significantly affect a development of lactic acid bacteria that are present in the environment. In this regard, sodium chloride, sodium acetate, disubstituted ammonium citrate, sodium citrate, sources of manganese, magnesium, phosphorus, iron and other elements are used (Colombo et al., 2014). This fact requires a careful and focused approach in the selection process of raw ingredients for nutrient media preparation (Hayek et al., 2019). Therefore, a nutrient medium for cultivation of lactobacilli was enriched by a various ratios of lactose, sucrose and cystine with addition of skimmed milk up to 100% (Table 1).

Table 1. Composition of nutrient medium for lactobacteria cultivation.

No	Components	Nutrient Media		
		No. 1	No. 2	No. 3
1	Lactose, g	–	5.0 ± 0.01	10.0 ± 0.02
2	Sucrose, g	–	15.0 ± 0.02	10.0 ± 0.01
3	Cystine, g	0.01 ± 0.005	0.01 ± 0.005	0.01 ± 0.005
4	Skimmed milk, cm^3	up to 100	up to 100	up to 100

Bifidobacteria are very demanding on composition of nutrient media, especially on easily digestible nitrogenous compounds, amino acids, carbohydrates, unsaturated fatty acids, vitamins and mineral elements (Roy, 2001). The components were selected for the production of a nutrient medium for bifidobacteria cultivation in the following ratio (mass %): glucose – 1.0-1.5, lactose – 1.0-1.5, cystine – 0.01-0.03, ammonium citrate – 0.2-0.4; potassium phosphate monosubstituted – 0.2-0.4, magnesium sulfate heptahydrate – 0.02-0.04, manganese sulfate tetrahydrate – 0.005-0.007, sodium phosphate bisubstituted – 0.2-0.4, microbiological agar – 0.2-2.5, sodium citrate – 0.5-0.8, distilled water – the rest. Distilled water was added up to 100% after dissolving the ingredients and then boiled. The pH was adjusted to 6.2-6.6, and the nutrient medium was filtered through a paper filter. The medium was sterilized and tested for sterility by keeping in a thermostat at a temperature of 37°C for 3-5 days (Table 2).

Table 2. Composition of nutrient medium for bifidobacteria cultivation.

No	Components	Nutrient Media	
		No. 1	**No. 2**
1	Peptone, g	5.0 ± 0.01	6.0 ± 0.02
2	Yeast autolysate, g	2.0 ± 0.03	3.0 ± 0.01
3	Glucose, g	1.0 ± 0.01	1.2 ± 0.01
4	Sucrose, g	1.0 ± 0.02	1.2 ± 0.03
5	Cystine, g	0.01 ± 0.01	0.02 ± 0.01
6	Ammonium Citrate, g	0.2 ± 0.01	0.3 ± 0.02
7	Potassium phosphate monosubstituted, g	0.2 ± 0.03	0.3 ± 0.02
8	Magnesium sulfate, g	0.02 ± 0.001	0.03 ± 0.002
9	Manganese sulfate, g	0.005 ± 0.001	0.006 ± 0.003
10	Sodium phosphate disubstituted, g	0.2 ± 0.01	0.3 ± 0.01
11	Microbiological agar, g	0.2 ± 0.02	2.0 ± 0.01
12	Sodium Citrate, g	0.5 ± 0.01	0.7 ± 0.04
13	Distilled water, cm^3	up to 100	up to 100

Thus, as a result of the studies the components of nutrient media were determined. They are sources of nitrogen, vitamins, carbohydrates, minerals, and the protein component for lactic acid bacteria.

The study of the growth properties of the nutrient medium for lactobacilli was the next stage of our work. Moreover, studies have shown that all samples of experimental nutrient media for cultivation of lactobacilli provided a higher level of accumulation of *Lactobacillus* spp. compared to the control nutrient medium (Table 3).

Table 3. Growth properties of culture media for the cultivation of *Lactobacillus* spp. (here and then n=3).

***Lactobacillus* strain**	**Nutrient medium**	**Growth, CFU/cm^3**
	No. 1	$7.1 \pm 0.23 \times 10^7$
	No. 2	$6.7 \pm 0.23 \times 10^8$*
Lactobacillus plantarum 7	No. 3	$8.8 \pm 0.19 \times 10^8$*
	No. 4	$8.9 \pm 0.17 \times 10^{10}$*
	control	$6.3 \pm 0.21 \times 10^7$
	No. 1	$7.3 \pm 0.20 \times 10^7$*
	No. 2	$8.9 \pm 0.24 \times 10^7$*
Lactobacillus casei 27	No. 3	$10.7 \pm 0.21 \times 10^8$*
	No. 4	$10.8 \pm 0.19 \times 10^9$*
	control	$7.5 \pm 0.23 \times 10^6$
	No. 1	$7.1 \pm 0.19 \times 10^7$*
	No. 2	$7.7 \pm 0.24 \times 10^8$*
Lactobacillus plantarum 7-317	No. 3	$8.7 \pm 0.18 \times 10^8$*
	No. 4	$9.4 \pm 0.22 \times 10^9$*
	control	$6.9 \pm 0.21 \times 10^6$

*$p<0.05$ compared to control

The most intensive growth of *Lactobacillus* spp. was observed on experimental nutrient media No. 3 and No. 4. Thus, the growth rate of *Lactobacillus plantarum 7* was $8.8 \pm 0.19 \times 10^8$ CFU/cm^3 on experimental nutrient medium No. 3 and $8.9 \pm 0.17 \times 10^{10}$ on medium No. 4. This index was $6.3 \pm 0.21 \times 10^7$ CFU/cm^3 on a control nutrient medium. A *Lactobacillus casei 27* culture also grew better on experimental nutrient media No. 3 and No. 4. A growth rate was $10.7 \pm 0.21 \times 10^8$ CFU/cm^3 and $10.8 \pm 0.19 \times 10^9$ CFU/cm^3 on listed media ($7.5 \pm 0.23 \times 10^6$ CFU/cm^3 on control medium). A *Lactobacillus plantarum 7-317* bacteria grew on developed nutrient media No. 3 and No. 4. Active accumulation of a significant number of viable cells was observed within the range of $8.7 \pm 0.18 \times 10^8$ CFU/cm^3 and $9.4 \pm 0.22 \times 10^9$ CFU/cm^3 respectively ($6.9 \pm 0.21 \times 10^6$ CFU/cm^3 on control medium). It should be noted that experimental nutrient medium No. 4 is the best option for cultivation of *L. plantarum 7*, *L. casei 27*, *L. plantarum 7-317* lactobacilli cultures compared to other experimental and control media. It should be noted that experimental nutrient medium No. 4 is the best option for cultivation of *L. plantarum 7*, *L. casei 27*, *L. plantarum 7-317* lactobacilli cultures compared to other experimental and control media.

The next stage of our work was to test the growth properties of the developed media for bifidobacteria *Bifidobacterium adolescentis 17* and *B. adolescentis 17-316* (Table 4).

Table 4. Growth properties of culture media for the cultivation of *Bifidobacterium* spp.

Nutrient medium	**Growth, CFU/cm^3**	
	Bifidobacterium adolescentis 17	***Bifidobacterium adolescentis 17-316***
No. 1	$4.5 \pm 0.22 \times 10^6$	$5.5 \pm 0.19 \times 10^6$
No. 2	$5.7 \pm 0.27 \times 10^7$*	$5.7 \pm 0.27 \times 10^7$*
No. 3	$6.4 \pm 0.23 \times 10^8$*	$5.9 \pm 0.23 \times 10^8$*
control	$4.1 \pm 0.19 \times 10^6$	$5.2 \pm 0.17 \times 10^6$

Studies have shown that nutrient medium No. 3 is optimal for the cultivation of microorganism *B. adolescentis 17* and *B. adolescentis 17-316*. It provides a high yield of a bacterial mass of these bifidobacteria in the range of $6.4 \pm 0.23 \times 10^8$ and 5.9

± 0.23 × 10^8 respectively. The next step in our work was to determine the optimal conditions for the cultivation of lactobacteria and bifidobacteria. The conditions under which microorganisms are cultivated have played a decisive role in their biological activity manifestation, including those used in the composition of probiotic preparations (Ram & Chander, 2003). The acidity and alkalinity of nutrient media are the most important properties of these media that influences on the life processes of microorganisms. It is expressed as the negative logarithm of hydrogen ions concentration (pH). The pH value influences on a dissociation of acids in the nutrient medium, solubility of nutrients and their transport into the cell, activity of enzymes etc. Thus, this factor determines the possibility of the growth of microorganisms on a nutrient medium that is optimal by its composition (Waddington et al., 2010).
We have studied a growth of *L. plantarum 7*, *L. casei 27*, *L. plantarum 7-317*, *B. adolescentis 17* and *B. adolescentis 17-316* strains at various pH and temperature. It was found that there is an inverse correlation between the pH value of nutrient medium and the number of lactic acid bacteria that grow on it. A high pH value accompanied by the presence of a small number of these bacteria (Table 5).

Table 5. Effect of nutrient medium Ph on the growth of lactic acid bacteria.

pH	Growth, × 10^6 CFU/cm^3	
	Control	Experiment
5.5	13.7 ± 0.15	14.1 ± 0.14
6.0	14.4 ± 1.16	14.5 ± 1.10
6.5	23.2 ± 1.70	24.0 ± 1.16*
7.0	24.3 ± 1.30	25.0 ± 1.14*
7.5	22.5 ± 1.10	23.0 ± 1.01*
8.5	10.1 ± 0.50	12.0 ± 4.10

As a result of statistical analysis, the most intensive growth of microorganisms was observed on nutrient medium at pH 6.5-7.5. Thus, the maximum accumulation of probiotic cultures was observed at pH 7.0 (control – 24.3 ± 1.30 × 10^6 CFU/cm^3; experiment – 25.0 ± 1.14 × 10^6 CFU/cm^3), and the number of microbial cells was 14.1 ± 0.14 × 10^6 CFU/cm^3 and 14.5 ± 1.10 × 10^6 CFU/cm^3 at pH 5.5-6.0 respectively. The lowest concentration of microorganism 12.0 ± 4.10 × 10^6 CFU / cm^3 was observed at pH 8.5. We also studied the optimal temperature conditions for the cultivation of lactic acid bacteria.
The cultivation of lactic acid bacteria was carried out at temperatures from 30°C to 50°C (Table 6). Studies have shown that the best growth of lactic acid bacteria was at a temperature of 37.0-40.0 ± 0.5°C. Growth of cultures was low and ranged from 9.00 ± 0.87 to 1.20 ± 0.05 × 10^6 CFU/cm^3 at a temperature of 30.0, 45.0 and 50.0 ± 0.5°C. Consequently, an optimal pH value is 7.0, and incubation temperature of an inoculum is 37.0 ± 0.5°C at different cultivation modes of lactic acid bacteria. The results of these studies make it possible to improve the cultivation technology lactobacilli and bifidobacteria that based on the optimal growth substrate. This will allow to apply the obtained results for a manufacture of innovative probiotic preparations.

Table 6. Effect of temperature on the growth of lactic acid bacteria.

Temperature,°C	Growth, × 10^6 CFU/cm^3	
	Control	Experiment
30.0 ± 0.5	14.5 ± 1.77	6.25 ± 0.15*
37.0 ± 0.5	24.7 ± 0.36	25.00 ± 0.34*
40.0 ± 0.5	23.5 ± 0.92	24.00 ± 0.87*
45.0 ± 0.5	8.05 ± 0.92	9.00 ± 0.87
50.0 ± 0.5	9.05 ± 0.06	1.20 ± 0.05

Conclusion

A nutrient medium for the cultivation of lactobacilli is proposed. A new nutrient medium has the following composition: lactose – 10.0-15.0%, sucrose – 5.0-10.0%, cystine – 0.01%, skim milk – up to 100.0%. The nutrient medium optimal for the accumulation of bifidobacteria had the following composition of components (mass %): peptone – 7.0 ± 0.01, yeast autolysate – 4.0 ± 0.01, glucose – 1.5 ± 0.02, sucrose – 1.5 ± 0.01, cystine – 0.03 ± 0.01, ammonium citrate – 0.4 ± 0.03, potassium phosphate monosubstituted – 0.4 ± 0.02, magnesium sulfate – 0.04 ± 0.002, manganese sulfate – 0.007 ± 0.002, sodium phosphate disubstituted – 0.4 ± 0.02, microbiological agar – 2.5 ± 0.04, sodium citrate – 0.8 ± 0.05, and distilled water – up to 100%. We determined that the optimal temperature for cultivation of lactic acid bacteria is 37.0 ± 0.5°C, and the pH value of the nutrient medium is 6.5-7.5.

References

Alayande, K. A., Aiyegoro, O. A., & Ateba, C. N. (2020). Probiotics in Animal Husbandry: Applicability and Associated Risk Factors. Sustainability, 12, 1087. doi: 10.3390/su12031087

Ben Salah, R., Trabelsi, I., Ben Mansour, R., Lassoued, S., Chouayekh, H., & Bejar, S. (2012). A new *Lactobacillus* plantarum strain, TN8, from the gastro intestinal tract of poultry induces high cytokine production. Anaerobe, 18 (4), 436-444. doi: 10.1016/j.anaerobe.2012.05.001

Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic Mechanisms of Action. Annals of Nutrition and Metabolism, 61, 160-174. doi: 10.1159/000342079

Celiberto, L. S., Bedani, R., Rossi, E. A., & Cavallini, D. C. U. (2017). Probiotics: The scientific evidence in the context of inflammatory bowel disease. Critical reviews in food science and nutrition, 57(9), 1759-1768. doi: 10.1080/10408398.2014.941457

Chaucheyras-Durand, F., & Durand, H. (2010). Probiotics in animal nutrition and health. Beneficial microbes, 1(1), 3-9. doi: 10.3920/BM2008.1002

Chiang, M. L., Chen, H. C., Chen, K. N., Lin, Y. C., Lin, Y. T., & Chen, M.-J. (2015). Optimizing production of two potential probiotic lactobacilli strains isolated from piglet feces as feed additives for weaned piglets. Asian-Australasian journal of animal sciences, 28(8), 1163-1170. doi: 10.5713/ajas.14.0780

Chiu, Y. H., Lin, S. L., Tsai, J. J., & Lin, M. Y. (2014). Probiotic actions on diseases: implications for therapeutic treatments. Food and function, 5(4), 625-634. doi: 10.1039/c3fo60600g
Colombo, M., de Oliveira, Z. A. E., de Carvalho, F. A., & Nero, L. A. (2014). Development of an alternative culture medium for the selective enumeration of *Lactobacillus* casei in fermented milk. Food Microbiology, 39, 89-95. doi: 10.1016/j.fm.2013.11.008
Gadde, U., Kim, W. H., Oh, S. T., & Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. Animal health research reviews, 18(1), 26-45. doi: 10.1017/S1466252316000207
Gujvinska, S. A., & Paliy, A. P. (2018a). Determination of antagonistic and adhesive properties of lactobacterium and bifidobacterium. Mikrobiolohichnyi Zhurnal, 80(1), 36-44. doi: 10.15407/microbiolj80.01.036
Gujvinska, S. A., & Paliy, A. P. (2018b). Biological properties of lactobacilli and bifidobacteria. Veterinary biotechnology. Bulletin, 32(1), 91-97. (In Ukrainian)
Gujvinska, S. A., Paliy, A. P., Dunaeva, O. V., Paliy, A. P., & Berezhna, N. V. (2018). Biotechnology production of medium for cultivation and lyophilization of lactic acid bacteria. Ukrainian Journal of Ecology, 8(2), 5-11. doi: 10.15421/2018_302
Hadzevych, O. V., Paliy, A. P., Kinash, O. V., Petrov, R. V., & Paliy, A. P. (2019). Antibiotic resistance of microorganisms isolated from milk. World of Medicine and Biology, 3(69), 245-250. doi: 10.26724/2079-8334-2019-3-69-245-250
Hayek, S. A., Gyawali, R., Aljaloud, S. O., Krastanov, A., & Ibrahim, S. A. (2019). Cultivation media for lactic acid bacteria used in dairy products. Journal of Dairy Research, 86(4), 490-502. doi: 10.1017/S002202991900075X
Herawati, V. E., Pinandoyo, P., Rismaningsih, N., Darmanto, D., Hutabarat, J., & Radjasa, O. K. (2020). The effect of probiotic bacteria in culture media using organic fertilizer for population density, biomass production and nutrient quality of Phronima sp. as natural feed. Aquaculture Research, 51(2), 836-843. doi: 10.1111/are.14433
Hossain, M. I., Sadekuzzaman, M., & Ha, S. D. (2017). Probiotics as potential alternative biocontrol agents in the agriculture and food industries: A review. Food research international, 100(1), 63-73. doi: 10.1016/j.foodres.2017.07.077
Irkitova, A.N., Matsyura, A.V. (2017). Ecological and biological characteristics of *Lactobacillus acidophilus*. Ukrainian Journal of Ecology, 7(4), 214–230.
Irkitova A.N., Grebenshchikova A.V., Matsyura A.V (2018). Antagonistic activity of Bacillus subtilis strains isolated from various sources. Ukrainian Journal of Ecology, 8(2), 354-364 doi: 10.15421/2018_354.
Kneifel, W. (2000). In vitro growth behaviour of probiotic bacteria in culture media with carbohydrates of prebiotic importance. Microbial Ecology in Health and Disease, 12:1, 27-34, doi: 10.1080/089106000435563
Markowiak, P., & Śliżewska, K. (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens, 10:21. doi: 10.1186/s13099-018-0250-0
Nwamaioha, N. O., & Ibrahim, S. A. (2018). A selective medium for the enumeration and differentiation of *Lactobacillus* delbrueckii ssp. Bulgaricus. Journal of Dairy Science, 101, 4953-4961. doi: 10.3168/jds.2017-14155
Odegov, N.I., Dorofeev, R.V., Irkitova, A.N., Funk, I.A., Orlova, T.N., Matsyura, A.V. (2017). Diversity of phage associations homologous to lactic acid bacteria. Ukrainian Journal of Ecology, 7(4), 197–206.
Ozen, M., & Dinleyici, E. C. (2015). The history of probiotics: the untold story. Beneficial microbes, 6(2), 159-165. doi: 10.3920/BM2014.0103
Paliy, A. P., Gujvinska, S. A., Livoshchenko, L. P., Nalivayko, L. I., Livoshchenko, Ye. M., Risovaniy, V. I., Dubin, R. A., Berezhna, N. V., Palii, A. P., & Petrov, R. V. (2020). Specific composition of indigenous microflora (*Lactobacillus* spp., Bifidobacterium spp., Lactococcus spp.) in farm animals. Ukrainian Journal of Ecology, 10(1), 43-48. doi: 10.15421/2020_7
Parvez, S., Malik, K. A., Kang, S. Ah., & Kim, H.-Y. (2006). Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology, 100(6), 1171-1185. doi: 10.1111/j.1365-2672.2006.02963.x
Patel, R. M., Myers, L. S., Kurundkar, A. R., Maheshwari, A., Nusrat, A., & Lin, P. W. (2012). Probiotic bacteria induce maturation of intestinal claudin 3 expression and barrier function. The American journal of pathology, 180 (2), 626-635. doi: 10.1016/j.ajpath.2011.10.025
Ram, C., & Chander, H. (2003). Optimization of culture conditions of probiotic bifidobacteria for maximal adhesion to hexadecane. World Journal of Microbiology and Biotechnology, 19, 407-410. doi: 10.1023/A:1023946702949
Roy, D. (2001). Media for the isolation and enumeration of bifidobacteria in dairy products. International journal of food microbiology, 69(3), 167-182. doi: 10.1016/s0168-1605(01)00496-2
Sánchez, Ó. J., Barragán, P. J., & Serna, L. (2019). Review of *Lactobacillus* in the food industry and their culture media. Revista Colombiana de Biotecnología, 21(2), 63-76. doi: 10.15446/rev.colomb.biote.v21n2.81576
Senz, M., Van, L., Bader, J., & Stahl, U. (2015). Control of cell morphology of probiotic *Lactobacillus acidophilus* for enhanced cell stability during industrial processing. International journal of Food Microbiology, 792, 34-42. doi: 10.1016/j.ijfoodmicro.2014.09.015
Süle, J., Kõrösi, T., Hucker, A., & Varga, L. (2014). Evaluation of culture media for selective enumeration of bifidobacteria and lactic acid bacteria. Brazilian journal of microbiology, 45(3), 1023- 1030. doi: 10.1590/s1517-83822014000300035
Uzunova-Doneva, T., & Donev, T. (2005) Anabiosis and conservation of microorganisms. Journal of culture collections, 4(1), 17-28.
Waddington, L., Cyr, T., Hefford, M., Hansen, L. T., & Kalmokoff, M. (2010). Understanding the acid tolerance response of bifidobacteria. Journal of Applied Microbiology, 108, 1408-1420. doi: 10.1111/j.1365-2672.2009.04540.x
Wang, B., Xu, H., Wei, H., Zeng, Z., & Xu, F. (2015). Oral administration of Bifidobacterium bifidum for modylating microflora, acid and bile resistance, and phylosiological indices in mice. Canadian journal of microbiology, 61(2), 155-163. doi: 10.1139/cjm-2014-0694
Yang, E., Fan, L., Yan, J., Jiang, Y., Doucette, C., Fillmore, S., & Walker, B. (2018). Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. AMB Express, 8(1), 10. doi:10.1186/s13568-018-0536-0
Yirga, H. (2015) The Use of Probiotics in Animal Nutrition. Journal of Probiotics & Health, 3(2), 132. doi: 10.4172/2329-8901.1000132
Zhu, G., Ma, F., Wang, G., Wang, Y., Zhao, J., Zhang, H., & Chen, W. (2018). Bifidobacteria attenuate the development of metabolic disorders, with inter-and intra-species differences. Food & Function, 9, 3509-3522. doi: 10.1039/c8fo00100f.

Citation:
Paliy, A.P., Gujvinska, S.A., Rodionova, K.O., Alekseeva, N.V., Ponomarenko, O.V., Alrawashdeh, M.S., Yeletskaya, T.A., Ponomarenko, G.V., Kushnir, V.Yu., Palii, A.P. (2020). Enhanced cultivation technology for lacto- and bifidobacteria. *Ukrainian Journal of Ecology, 10*(3), 83-87.