SELECTING THE OPTIMAL ENVIRONMENT FOR CULTIVATION OF LACTOBACILLUS RHAMNOSUS GG BIOMASS

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Introduction. Today, 65% of the healthy food market is occupied by products and supplements aimed at normalizing the digestive system, particularly probiotics. Probiotics are also used to reduce the negative consequences of antibiotic therapy, which is currently in high demand. In Ukraine, for example, 2.6% of the population received antibiotic therapy in 2020. Lactobacillus species are often used as probiotic microorganisms, but due to their demanding nutritional requirements, cultivating these bacteria can be quite expensive [1].

The aim of our work is to select the most optimal environment for the growth of lactobacilli, particularly *Lactobacillus rhamnosus* GG (LGG).

Materials and methods. Literary sources devoted to the conditions and environments for cultivating LGG that provide stable probiotic properties have been analyzed, and their cost and economic feasibility have been calculated.

Results and discussion. The selection of a nutrient medium is one of the main factors that determine the possibility of industrial cultivation of microorganisms. Currently, the optimal medium for cultivating lactobacilli is the MRS medium, but using this medium during the production of probiotics can be quite expensive. Therefore, there is an urgent need to reduce the cost of existing probiotic cultivation technologies by introducing new physiologically efficient and relatively inexpensive nutrient media into production practice.

L. rhamnosus GG is a type of gram-positive bacteria that falls under the Lactobacillus genus and Lactobacillaceae family. It is naturally found in humans and has notable antagonistic activity against pathogenic and opportunistic microorganisms. Moreover, it exhibits resistance to the acidic and enzymatic environments of the gastrointestinal tract, possesses strong adhesion properties, stable genetic traits, and is considered a GRAS organism. Studies have shown that L. r*hamnosus* GG can alleviate symptoms of various gastrointestinal disorders such as diarrhea, irritable bowel syndrome, and inflammatory bowel disease. It can also improve the symptoms of respiratory infections and allergies. Moreover, it has been suggested that this strain can potentially prevent the development of certain types of cancers.The primary criterion for selecting this microbe was its scientifically proven ability to reduce the duration of rotavirus infection by stimulating the synthesis of immunoglobulin A. Additionally, it has no antigenic properties towards the human body [2, 3].

The comparative characteristics of the media for *L. rhamnosus* GG cultivation are presented in Table 1. According to the analyzed sources, the shortest cultivation time is observed in the medium from article [4], while the highest cell concentration is achieved in the medium mentioned in the article [5]. But in order to finally choose the most optimal environment, you need to calculate its cost.in Table 2.

Based on the information provided, it appears that the optimal medium for producing the target product is medium №1. This is because the conditional cost of biomass produced on this medium is the lowest at 4.34 UAH/g, and the biomass

accumulation rate is the fastest at 0.5 g/hour. Additionally, medium №1 allows for improved cell viability after lyophilization, which is a process that significantly increases the shelf life of bacterial biomass and ensures the stability of its characteristics.

Table 1. Features of *Lactobacillus rhamnosus* GG cultivation on a growth substrate mixture

Nutrient medium composition:		**Cultivation duration, h**	**Cell concentration (CFU/ml)**	**Features of the biosynthesis process**	**Total cost of 1 liter of medium, UAH***
Components	**concentration, g/L**				
1	2	3	4	5	6
Glucose K_2HPO_4 Yeast extract Peptone KH_2PO_4 $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 4H_2O$ Tween 80,	20 11,3 10 10 4,6 0,2 0,05 0,1	12	10^9	Periodic cultivation at 37°C (based on inoculum cultivation data) [3].	26,06
Sweet potato Proteose pepton Beef extract Yeast extract CH3COONa Tween 80 Na2HPO4 K2HPO4 NH4C6H5O7 MgSO4*7H2O MnSO4*5H2O L-Cysteine	450 8 8 8 5 1 мл 2 2 2 0,1 0,05 1	24	$5,6 \times 10^{10}$	At a temperature of 37°C, with an initial pH range of 6.37-6.54. [4].	127,457

Note. * - Prices are given as of February 2022.

Table 2. Conditional cost of 1 g of *Lactobacillus rhamnosus* GG cells synthesized on a mixture of growth substrates

The nutrient medium	**Biomass concentration, g/L**	**Cultivation duration, h**	**Amount of biomass formed per hour, g/hour**	**Cost of 1 L of medium, UAH/L**	**Conditional cost of 1 g of target product, UAH/g**
1	2	3	4	5	6
1 [4]	6	12	0,5	26,06	4,34
2 [5]	6	24	0,25	127,457	21,243

It is important to note that while the cost of biomass production is a significantfactor in determining the optimal medium, other factors such as product quality and stability should also be taken into account. The fact that medium №1 allows for improved cell viability after lyophilization is an important consideration in ensuring the quality and stability of the final product [4].

The removal of water during lyophilization inhibits microbial growth, thus improving the shelf-life of the product. Lyophilized probiotic biomass can be easily rehydrated and incorporated into a variety of products, such as dietary supplements, functional foods, and pharmaceuticals. Also, reduced weight and volume of lyophilized probiotic biomass make it easier and more cost-effective to transport and low-temperature process of lyophilization helps to preserve the viability and functionality of probiotic cells [4, 6].

Conclusions. Having analyzed the data obtained, we will stop our choice on medium №1 Firstly, it is more cost-effective than other mediums, which is an important factor in commercial production. Secondly, the use of this medium allows for an increase in the survival rate of lactobacilli during the lyophilization process up to 80%, which is crucial for maintaining the potency of the final product.Specifically, probiotics in the form of lyophilizate are included in the composition of many drugs, dietary supplements, or food products [4].

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