Microbiological synthesis optimization of probiotic microorganisms *Lactobacillus plantarum* using combined nutrient media based on AIC secondary raw materials

Irina Gagarina[1*], *Irina* Gorkova[1], *Ekaterina* Kostromicheva[1], *Anna* Gavrilova[1], and *Sergey* Polekhin[1]

[1]Oryol State Agrarian University named after N.V. Parakhin, 302019, Russia, Oryol, Generala Rodina st., 69

Abstract. The modern agro-industrial complex of the Russian Federation produces a significant number of by-products containing valuable nutrients. The article considers the possibilities of using secondary raw materials of agro-industrial sector in biotechnology industry. The role of waste processing of agricultural raw materials in optimization of lactobacilli cultivation process has been studied. Experimental studies of growth dynamics of probiotic microorganisms *Lactobacillus plantarum* on complex nutrient media using food and agriculture production waste were carried out. The optimal composition of the nutrient medium for lactobacilli growth and the time of microorganisms cultivation corresponding to the maximum increase of biomass were determined. Comparative analysis of media components for growing *Lactobacillus plantarum* according to the growth index of probiotic microorganisms was carried out. The growth stimulating role of such substrates for cultivation as beet pulp and corn gluten has been established.

1 Introduction

One of the most important directions of the state industrial policy of Russia is creating conditions for expanding the raw material base of the domestic economy, reducing material losses, reducing the level of environmental pollution. A key role in the transition to environmentally sustainable development should be the transition from an extensive export-commodity model of economic development to a model of sustainable development, economy ecologization [1]. In this regard, the secondary raw materials use may be considered as one of the means of material resources reproduction.

The introduction of ecologization and production recycling principles finds wide prospects in the agro-industrial complex. This sector of the economy is a waste-intensive industry. Production of the main product is associated with the formation of a large quantity

* Corresponding author: i-gagarina@list.ru

of by-products, and the development and implementation of zero-waste technologies is the most promising way to improve production profitability [2,3].

Currently, the agro-industrial complex does not fully utilize secondary raw materials. The output of the main product is sometimes 15 -30% of the raw material mass [2,4]. Due to the development of biotechnological recycling principles, AIC waste can be considered as secondary raw materials for recycling industries. The recycling process has several important aspects, both economic and social, not only for the enterprises of the industry, but also for the country as a whole. First - deepening the agricultural raw materials recycling and ensuring low-waste production, and hence the profits of enterprises. Secondly - improving the environmental situation of the regions by reducing harmful discharges of enterprises. And thirdly - an increase in the production of valuable feed of biological origin for livestock, poultry, animal husbandry, service dog breeding and pets [5].

Recycled agro-industrial complex waste can be used not only in agriculture but also in other industries such as food, microbiological, biotechnology, pharmaceutical, chemical, etc. One of the directions of agro-industrial waste recycling is the use of secondary raw materials as components of nutrient microbiological media for the cultivation of probiotic microorganisms. Replacing synthetic media with combined media will significantly reduce the cost of the final product by reducing the number of expensive compounds in the nutrient substrate, such as sources of nitrogen, carbohydrate, minerals and vitamins. Probiotic additives have been widely used in feed production. They include microorganisms of natural animal microflora, such as lactobacilli, bifidobacteria, some streptococci, clostridia, eubacteria, etc. Intestinal microflora regulates the fermentation processes of feed compounds, induction of anatomical and physiological changes in the structure of the cell wall of the intestine, increasing the resistance of animals to enteropathogenic bacteria [10].

The modern concept of rational animal feeding provides for the use of full-fledged feed, ensuring optimal use of genetic potential of animal productivity and obtaining quality products from them [6,13]. Due to the introduction of the ban on the use of antibiotics in feed, the role of probiotic supplements has significantly increased [12]. Probiotics have a useful effect on the host animal by improving its microbiome by inhibiting the development of pathogens due to competition for substrates [7,9,14]. Probiotic supplements can not only improve the functions of natural animal microflora of highly productive breeds, but become an additional source of amino acids and vitamins, improving the quality of the final products.

In this paper we considered the possibilities of using food industry waste as raw materials for the cultivation of probiotic microorganisms *Lactobacillus plantarum*. Lactic acid bacteria are most commonly used as probiotics. They can be isolated from animals, plants and the environment [11]. A study of the nutrient medium composition effect on the yield of the cultural *Lactobacillus plantarum* was conducted.

At present, the use of secondary resources generated by the processing of renewable agricultural plants is becoming more urgent. Full use of secondary resources of recycling plant raw materials by biotechnological methods is economically expedient and promising for the biotechnology industry [8]. In order to optimize and reduce the cost of the nutrient environment, we conducted an experimental study with the addition of waste from starch, sugar, flour milling production.

2 Materials and methods of research

The chosen object of the study was laboratory culture of microorganisms *Lactobacillus plantarum*. Waste from the food recycling industry was used as additional elements of nutrient media: corn gluten and germ, beet pulp, wheat bran and potato pulp. Cultivation of lactobacilli was carried out by a deep method. Gauze medium was used as control medium

for cultivation; it consisted of g/l: hydrolysate of soybean flour; $MgSo_4$-0.02 g/l, NaCl-0.02g/l, $CaCl_2$- 0.00075mg/L, $FeSO_4$-0.001 g/l. The number of viable microorganisms was determined by direct counting method in Goryaev's counting chamber. Cultivation was carried out for 72 hours. The value of CFU/ml in the seed material was 2.1.

3 Results and Discussion

In order to optimize and reduce the cost of the nutrient environment, we conducted an experimental study on the cultivation of *Lactobacillus plantarum* in complex media with addition agricultural recycling by-products. The experiment options are presented in table1.

Table 1. Components of experimental nutrient media for the cultivation of *Lactobacillus plantarum*

Components of experimental nutrient media	Concentration of nutrient media components, ml/l, g/l				
	option 1	option 2	option 3	option 4	option 5
Yeast extract	0.2	0.2	0.2	0.2	0.2
Glucose syrup	2	2	2	2	2
Ascorbic acid	0.4	0.4	0.4	0.4	0.4
Potassium dehydroorthophosphate	0.2	0.2	0.2	0.2	0.2
Potato pulp	15	-	-	-	-
Beet pulp	-	9.2	-	-	-
Corn gluten	-	-	10	-	-
Corn grain germ	-	-	-	9.8	-
Wheat bran	-	-	-	-	28.4

The conducted studies showed that the maximum accumulation of lactobacilli culture falls on 36 hours of experiment on all studied media including control (Fig. 1). Figure 1a shows the dynamics of accumulation of *Lactobacillus plantarum* microorganisms on the control nutrient medium. The stationary growth phase lasted from 24 to 36 hours. The number of colony-forming bacteria in 1 ml of medium at 36 hours of cultivation in the control variant was 6.4 CFU/ml. Thus, biomass tripled. At the end of the experiment (72 hours), the number of viable cells decreased to 3.9 CFU/mL.

All experimental environments included yeast extract (0.2 g/l) as a source of amino acids, vitamins and minerals, as well as additional raw materials in the form of industries' waste. The first experimental medium included dry potato pulp at a concentration of 15 g/l. Potato pulp is a waste of starch production. It contains fiber, raw protein, residual amount of starch and a number of macro and micronutrients. In this regard, pulp is of interest as an alternative raw material for the cultivation of microorganisms. The linear growth phase in this experiment lasted up to 24 hours, stationary - from 24 to 36 hours (Figure 1b). The maximum amount of biomass amounted to 11.5 CFU/ml. The increase was 81.7% compared to the initial concentration. This indicator exceeded the control by 1.8 times.

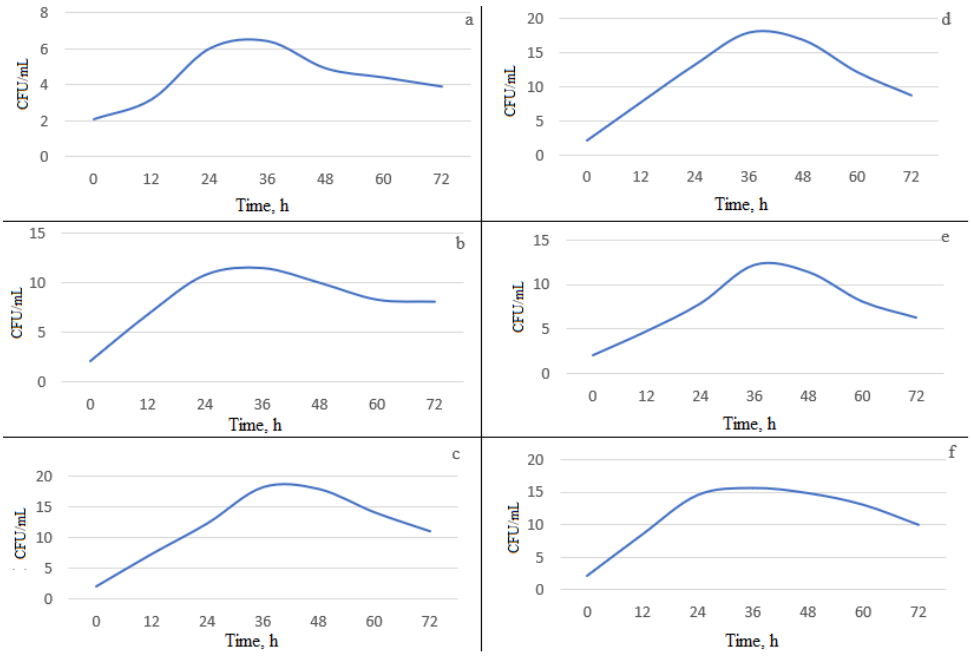

Fig. 1. Accumulation dynamics of *Lactobacillus plantarum* in the process of deep cultivation on various nutrient media: a - control, b - potato pulp, c - beet pulp, d — corn gluten, e - corn germ, f - wheat bran.

The second experimental medium contained beet pulp 9.2 g/l. The bulk mass of pulp is pectin substances, hemicellulose. The protein accounts for about 8%, fiber - about 22%. Figure 1c shows that the presence of beet pulp in the medium contributes to an increase in the period of linear growth up to 36 hours. The maximum accumulation of biomass *Lactobacillus plantarum* in this experiment amounted to 18.3 CFU/ml. This indicator is higher than the control by 2.9 times. Biomass increased by 88.5% in 36 hours of cultivation.

The third experimental medium included corn gluten (10 g/l). Gluten is a by-product in starch production. It contains protein, starch, fiber, lipids, macro- and micronutrients. This combination of components makes gluten a promising source of raw materials for the cultivation of microorganisms. Figure 1d shows the effect of gluten on *Lactobacillus plantarum growth dynamics*. It was found that the maximum accumulation of bacteria cells was 17.9 CFU/mL, which exceeds the control by 2.8 times. At the end of the experiment this indicator amounted to 8.7 CFU/mg. The decrease in growth dynamics was observed after 48 hours of cultivation.

The following nutrient medium contained crushed corn germ at a concentration of 9.8 g/l, a by-product of corn processing into starch or cereal. This component contributes to an increase in the nutrient content of lipids, proteins, fiber and water-soluble carbohydrates. In the experiment with corn germ (Fig. 1e), the largest number of bacteria cells was 12.2 CFU/ml at 36 hours of cultivation. The control values were exceeded by 1.9 times. The die-off phase was fixed after 48 hours of cultivation. In the option with gluten and corn germ, the stage of linear growth smoothly passes into stationary.

A valuable nutritional component in the cultivation of microorganisms is wheat bran. They contain up to 15.4% proteins, 8.5% fiber, 4% lipids. In the fifth experimental nutrient medium, wheat bran was contained in the amount of 28.4 g/l. The results of the experiment

are shown in Figure 1f. The maximum accumulation of biomass *Lactobacillus plantarum* in this experiment was 15.7 CFU. This indicator is higher than the control by 2,4 times. Biomass increased by 86.6% in 36 hours of cultivation.

Thus, the conducted studies showed the feasibility of using complex nutrient media based on yeast extract and secondary raw materials of agro-industrial complex.

4 Conclusions

1. It has been shown that the use of a nutrient medium based on yeast extract in combination with one of the complex organic raw materials such as potato pulp, beet pulp, corn gluten, corn grain germ or wheat bran increases the yield of probiotic microorganisms *Lactobacillus plantarum* in deep cultivation compared to the control medium based on soybean hydrolysate.
2. As a result of the conducted studies, the optimal composition of the experimental nutrient medium for growing *Lactobacillus plantarum*has been established. Maximum yield is marked on a medium containing beet pulp and corn gluten. It was found that when using a substrate based on yeast extract and beet pulp, the accumulation of biomass increases by 2.9 times; on the basis of yeast extract and corn gluten - by 2.8 times.
3. The optimal time of *Lactobacillus plantarum* cultivation was established as 36 hours, which corresponds to the maximum accumulation of lactobacillus culture on all studied media including control.

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