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THE NEW EFFICIENCY OF THE «SRMP» – LISTERIAS GROWTH-PROMOTING FACTOR DURING FACTORY CULTIVATION

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ABSTRACT

Despite the established technological regulations and clearly tried-and-true vaccine production mechanisms, there are certain difficulties both in the cultivation process and at the stage of freeze-drying, including" dry live vaccine from the "AUF" strain against the farm animals' listeriosis". Listeria growth-promoting factor "SRMP" was tested on the quails' egg embryonic tissue at different stages of preparation of the vaccine against the farm animals' listeriosis. The addition of 1% SRMP to the Hottinger's agar was effective at all the factory cultivation stages, and the mass of the *Listeria monocytogenes*, cultivated in the fermenter, increased by 25%. The addition of 1% SRMP to the drying environment of the vaccine against the farm animals' listeriosis enabled the microorganisms survival increase after lyophilization by 13% compared to the factory vaccine. Even after 12 months of vaccine storage, the *Listeria* survival rate in the vaccine with 13.3% SRMP was higher than in the factory vaccine.

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Introduction

Listeriosis is an infectious disease caused by *Listeria* bacteria; it causes considerable damage to the livestock and represents a serious threat to the human health [1-3].

As of today, people make specific prevention to control the listeriosis, which is done by using inactivated and live vaccines [1, 4].

Despite the advantages of the dry live vaccines, there are a number of unresolved production issues, including a vaccine against the farm animals' listeriosis. Thus, Pavlenko [5] paid particular attention to the issue of the instability reduction and the bacterial mass accumulation in the dry live vaccine of low virulent strain "AUF" manufacture process, for the farm animals' listeriosis prevention. He claimed it is connected with the non-standard nutrient mediums and meat hydrolyzates. Besides, Pavlenko noted that the instability of dry drugs is due to the reduction of viable microorganisms in the freeze-drying process and the vaccines storage.

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According to some authors, the instability issue and the reduction of bacterial mass yield might be solved by using the growth-promoting factor, which is required for microbial cells in small amounts, because it still enables a high reproduction rate and biomass accumulation [6-9].

Considering Listeria metabolic needs, we developed a new Listerias growth-promoting factor "SRMP based on quails' embryonic-egg mass and rational biotechnological manipulation". Its production methods enabled the creation of a unique complex of biologically active substances, such as peptides of various lengths, including low molecular weight, as well as nucleic acid fragments, which, according to some researchers, play an important role in microbial cells [1, 10-12]. Moreover, according to Ergun, (1982/1983) peptides of different lengths have specific protective properties (A. Ergun, 1982/1983), and Mority assumed that they prevent damage to cells during freezing, drying, and storage of the dry drugs [13, 14].

The aim of this research was to study the efficiency of SRMP when it is used in the cycle of making a vaccine against the farm animals' listeriosis.

Material and Methods

The experiment was carried out at Federal Government Enterprise (FGE) "Stavropol Biofactory". The strain was obtained from the Stavropol Biofactory archive. The *Listeria monocytogenes*, strain «AUF», sub-cultured in a tube containing semi-fluid agar, was cultured in a 100ml-vial with Hottinger's agar and incubated at 37±1°C for 24 hours. Then 10 ml of the culture was taken from the vial and cultured in the 100ml-vials containing Hottinger's agar and 1% SRMP. The control sample was Listeria, cultivated in the nutrient medium without any additives. The seedlings were incubated at 37±1°C for 24 hours.

To count the vials' colonies, the samples were taken from the vials and then they were diluted tenfold, after that, they were planted into 0.1 ml Hottinger's agar 10^{-6} dilutions.

The bacteria, cultivated in vials, with SRMP additives (the first generation), were added into vessels, which contained 12 L of manufacturing nutrient medium (the Hottinger's agar) and 1% SRMP, on the basis of 1 vial for 1 vessel to get the second generation. The control samples contained Listeria, cultivated in the nutrient medium without any additives. The cultures were incubated in a thermal room at 37±1 °C for 24 hours. To count the colonies, the samples were taken from the vials and then they were diluted tenfold, after that, they were cultured in 0.1 ml Hottinger's agar, 10^{-6} dilutions.

Before culturing in the-300 L fermenter, 1% SRMP (3 L) was added to the manufacturing nutrient medium. Then the pilot culture, grown with the use of the SRMP, was seeded from the cylinders into the fermenter with the amount of 10% of the nutrient medium. The control Listeria culture was added to a fermenter containing the same volume of the manufacturing nutrient medium. The SRMP growth-promoting effect was evaluated via centrifugation the media and weighting the Listeria biomass.

Bacterial mass, cultivated in fermenters, was used to produce the trial and control batch of anti-listeriosis vaccine for livestock.

The culture and morphological Listeria properties were evaluated at every stage of the study. The results were considered visually, via counting the grown colonies and also via smear microscopy, that were made from 24-hour-old cultures, grown at both thick and thin nutrient media and colored by the Gram staining.

To evaluate the SRMP effect on the *Listeria monocytogenes* viability during the cool dehumidification, an additional experimental vaccine batch was produced. The bacterial mass was diluted and thoroughly mixed in the 16-liter vessels with the multicomponent saccharose-gelatin-peptone-thiourea-glutamate dehumidification medium, which included 1% of the SRMP, on the basis of 100g biomass for every 1 L of the protective medium. Then the vaccine was packed into 10 cm^3 vials, where it was freeze-dried.

The vaccine against the farm animals' listeriosis production from the "AUF" strain and the quality control was performed according to the "Technological regulations for the production and control of dry live vaccine from the "AUF" strain against the farm animals' listeriosis " 00482861-0061-2009.

To determine the live Listeria concentration, 3 vials with dry vaccine were diluted to the original volume and the tenfold dilutions were prepared. For the trials, two vaccine dilutions were used: 10^{-8} and 10^{-9}. Five Petri dishes were assigned for each dilution containing 0.1 ml of the culture on the surface of the meat-peptone agar with 1% glucose and 20% inactivated serum of cattle. The cultures were incubated at 37±1°C for 72 h, and then the number of colonies in Petri dishes for each dilution were counted. The obtained values were summarized and divided by the number of dishes, identifying the average number of live bacteria in each dilution containing 0.1 cm^3 and determined the concentration of live bacteria in 1 cm^3 of the vaccine according to the following formula:

$$X = \frac{\frac{P}{g} + \frac{P_1 \times 10}{g_1}}{2} \times 10 \times 10^8$$

where X - The number of live bacteria in 1.0 cm^3;

P	-	The number of colonies in all dishes of 10^{-8} dilution;
g	-	number of dishes with the 10^{-8} dilution;
P_1	-	The number of colonies in all dishes of 10^{-9} dilution;
g_1	-	number of dishes with the 10^{-9} dilution;
2	-	The number of tested dilutions;
10	-	recalculation of the number of living bacteria in 1.0 cm^3;
10^8	-	The vaccine dilution percentage.

The dose of vaccine in the vial was determined by dividing the number of live Listeria by 1.0 cm^3 7.5 (7.5 billion live microbial cells comprise immunizing dose) and multiplying in the volume of the vaccine in the vial.
The GraphPad Prism 6.0 (USA) software was used for statistical analysis.

Results and Discussion

The drug "SRMP" was added to the production nutrient medium in the amount of 1% at all stages of cultivation of the vaccine strain. The optimal drug dosage was experimentally determined, using the Petri dishes with Hottinger's agar. The evaluation of SRMP's stimulating for the first generation production culture was shown that 1% SRMP addition to the Hottinger's agar enables stronger Listeria growth than the test medium growth. The number of colonies grown on the Petri dishes with Hottinger's agar, after the Listeria monocytogenes replanting, cultivated on the Hottinger's agar with the SRMP addition, exceeds the control by 2.3 times (table 1).

Table 1 - Hottinger's agar growth performance with the addition of SRMP (M±m)

Planting on the Hottinger's agar	The number of Petri dishes with the Hottinger's agar	The number of Listeria colonies grown from 10^{-6} dilution
Culture with the SRMP (1%) addition	5	42,8±0,1*
Control - without stimulants	5	18,3±0,1

Note: * (P<0.05) - are statistically significant results comparing to the control. The experiment has been replicated ten times.

To produce the second generation, we used the Listeria culture in vials, where 1 ml SRMP was added to 100 ml Hottinger's agar. The second-generation Listeria planted on the Hottinger's agar enabled to exceed the number of control colonies by 1.5 times (table 2).

Table 2 - Hottinger's agar growth performance with the SRMP addition for production of the second generation of *Listeria monocytogenes*, (M±m)

A variant of the second generation No.	The second generation on the Hottinger's agar	The number of Petri dishes with Hottinger's agar	The number of Listeria colonies grown from 10^{-6} dilution
1	The Hottinger's agar with SRMP (1%)	5	34,5±0,1*
2	Control – Hottinger's agar	5	22,7±0,2

Note:* (P<0.05) - are statistically significant results comparing to the control. The experiment was replicated ten times.

The second generation of the culture was planted into a fermenter containing the production nutrient medium with 1% SRMP.
The grown, cooled culture was centrifuged, collected, and weighed for the bacterial mass. Listeria biomass, grown in the production nutrient medium, served as the control, it was 600 g (2 g per 1 L), and the medium with SRMP addition - 800 g (2.7 g pr 1 L), i.e. 25% more.
From the research findings, it is evident that at all stages of Listeria cultivation during the production of vaccine against the farm animals' listeriosis, SRMP exerted a stimulating effect on the microorganism. The best stimulating effect was found for the first generation of Listeria. When using SRMP at the later cultivation stages, the Listeria biomass growth was somewhat lower than in the first generation. In our opinion, it is related to the reduced bacteria survival in suspension at high concentration due to the accumulation of the metabolites.
It was found (table 3) that by adding 1% SRMP to the drying production environment, the proportion of viable cells after freeze-drying was 80%, whereas in the control (production) series it was 67%, which means that adding SRMP to the drying environment of the vaccine increases the Listeria survival rate by 13%.

Table 3 – Listeria survival in the vaccine by adding SRMP to the drying environment before and after lyophilization

Substance name	Live Listeria concentration before lyophilization	Live Listeria concentration after lyophilization	Live Listeria concentration after 12 months vaccine storage
A pilot vaccine with the addition of the SRMP to the environment	45 billion/cm^3 (6 doses)	36 billion/cm^3 (4.8 dose)	30 billion/cm^3 (4 doses)
Factory vaccine (control)	45 billion/cm^3 (6 doses)	30 billion/cm^3 (4 doses)	21 billion/cm^3 (2.8 doses)

As the long-term vaccine storage also reduces the viability of the microorganisms, we compared the concentration of live Listeria in the vaccine, made with the SRMP addition to the drying environment and the control vaccine by the end of their shelf life, in 12 months. When storing the control vaccine series within 12 months the viability of Listeria monocytogenes decreased by 30%, whereas it was only 16.7% in the experimental series.

The results imply that the SRMP addition to the drying environment during the vaccine against the farm animals' listeriosis production increased the Listeria monocytogenes survival during long-term storage by 13.3% compared to the control vaccine.

Thus, SRMP, the new Listeria growth-promoting factor, is effective at different stages of the production of vaccine against the farm animals' listeriosis.

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