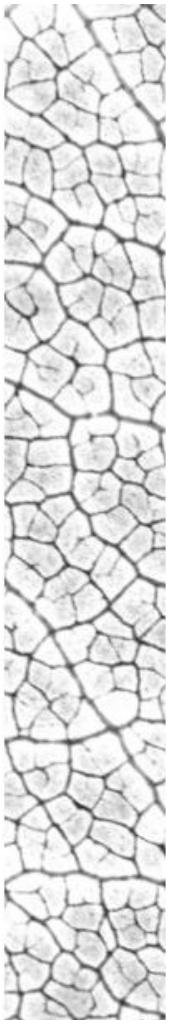

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Influence of lactic acid microorganisms on the formation of quality of dry sausages

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Abstract
In the production of meat products one of the promising areas is the use of microorganisms to improve the quality of the product. In this article the biotechnological potential of probiotic microorganisms Pediococcus pentosaseus, Staphylococcus xylosus, Staphylococcus carnosus, Bifidum longum 339M was studied. It was found that with an increase in salt concentration up to 4% the number of viable cells remains at a sufficiently high level and amounts to 10^{10}-10^{11} CFU/cm^3. With increasing doses of sodium nitrite, a higher antimutagenic activity of lactic acid bacteria was observed. The identified regularities serve as a scientific basis for reducing sodium nitrite toxicity.

Keywords: microorganism, sausage, antimutagenic activity, viable cells, revetrants

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INTRODUCTION

Lactic acid microorganisms are representatives of the normal microflora of the gastrointestinal tract of humans and animals. Despite their obvious practical value, they remain the least studied in terms of their use in the meat industry (Laranjo et al. 2019, Sydykova et al. 2019).

Meat products are one of the main sources of essential nutrients (minerals, vitamins, amino acids, etc.) in the human body (Kakimov et al. 2017, Okuskhanova et al. 2017). The theoretical prerequisite for the use of lactic acid bacteria for biotechnological processing of meat raw materials is a wide temperature range of growth (15-30) °C. There is evidence that they grow at lower temperatures (2-7) °C, the optimal pH value is 6.5-7.0, at a pH below 5.0 viability decreases, they are characterized by a sufficiently high resistance to salt. During the metabolism of lactic acid bacteria, lactic acid is formed, which has preservative properties. From other fermentations, it is distinguished by a high yield of ATP, the participation of some unique enzymes and reactions, they restore nitrites during the utilization of lactate, have high antimutagenic properties (Antara et al. 2019, Vorobieva and Abilev 2002). Despite the unique properties, lactic acid bacteria have not yet found wide application in the meat industry due to insufficient information about the mechanism of their manifestation in the meat substrate (Dumin et al. 2002, Kaldarbekova et al. 2019).

As for other properties – resistance to physical and chemical factors when cultured in the meat system, information about the manifestation of antimutagenic activity in the literature is not found.

Taking into account the above, it is of interest to develop new approaches to the selection of probiotic microorganisms for biotechnological processing of raw materials that meet the specific requirements of technological processes for the production of meat products (Platonova et al. 2019).

MATERIALS AND METHODS

To select the most promising strains of probiotic microorganisms, their biotechnological potential was studied. Various strains of mono and polycultures of lactic acid and bifidobacteria, as well as combined starter cultures were used for the research.

The objects of experimental research were ground beef, sausage minced meat, finished sausage products. The research materials were different strains of probiotic microorganisms, combined yeast *Pediococcus*

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pentosaseus, *Staphylococcus xylosus*, *Staphylococcus carnosus*.

According to the tasks, during the study of meat samples the following parameters were determined:

- the value of active acidity was determined by the potentiometric method;

- sodium chloride content was determined in the water extraction from the product by the Mohr method. The method is based on deposition of chlorine ions by silver ions in a neutral medium in the presence of potassium chromate as an indicator (GOST 9957-2015, 2015);

- The content of nitrite was determined by the method based on measuring the intensity of coloration formed by the interaction of sodium nitrite with sulfonamide and N (1-naphthyl)-ethylenediamine hydrochloride in protein-free filtrate (GOST 8558.1-2015, 2015);

- determination of the oxidation degree of fat was carried out using the method based on the oxidation of hydrogen iodine acid by peroxides contained in fat, followed by nitration of the released iodine with sodium thiosulphate;

- quantitative accounting of viable cells of lactic acid bacteria was determined using GMK-1 medium by the limit dilution method.

The medium was prepared according to the following method: dry medium (50 g) is brought in 1000 cm^3 of distilled water, heated to complete dissolution, in the presence of sludge is filtered, set the pH (7,1+0,2). The medium is poured into tubes of high column by (10±0.5) cm^3 and sterilized at 121 °C for (15±2) min. The ready medium before use should be regenerated in a water bath by boiling for 35 min. Then the medium is cooled down to (36+2) °C and used for sowing.

For qualitative accounting in sterile conditions, 1 cm^3 of starter is taken with a pipette and transferred to the first tube containing 10 cm^3 of medium. Then using new sterile pipette thoroughly stir the contents of the tube and 1 cm^3 mixture of it is transferred to the second tube, etc. In total, at least 15 dilutions are prepared.

Ready sowing is kept in a thermostat at (35±1) °C for 1-2 days. After the end of keeping the grown up colonies are counted, and the number of live cells in the last dilution is established (Khamagaeva et al. 2006, Zhuravskaya et al. 1985).

The mass fraction of fat was determined by chloroform extraction. The method is based on fat extraction from the product at constant shaking with subsequent separation of the extract and drying to a constant mass (Antipova et al. 2001).

Ames' test was used to determine antimutagenic activity, and Pediococcus pentosaseus test strain was used as a mutagenicity indicator. The strain carries a mutation of auxotrophicity by histidine and reverts to prototrophicity under the influence of mutagens that cause mutations such as base pair replacement. The principle of the method consists in the fact that under the influence of a mutagen on a selective medium grow reverts but histidine, the number of which determine the mutagenic effect. Accordingly, antimutagens reduce the number of induced revertants (Bondarenko and Savitskaya 2008).

The order of the experiment was as follows: in 2 ml of upper agar containing 0.5 mM histidine/biotic, 0.1 ml of fresh Pediococcus pentosaseus culture, 0.1 ml of tested mutagen and 0.1 ml of test sample antimutagen were added. The mixture was quickly stirred and poured onto the surface of a minimal agarized medium (lower agar) in Petri dishes. By intensive stirring, an uniform distribution of the upper agar on the lower agar surface was achieved. The cups were incubated for 48 h at 37°C. At the same time, positive control was given when there was no mutagen in the mixture but there was no antimutagen and negative control when there was no mutagen in the mixture but potential antimutagen was present. The total volume of the mixture was brought to 0.4 ml using 0.2 M phosphate buffer pH 7.4.

After the incubation of the culture, the number of revertants in the cups was calculated. The experiments were set in three repetitions and statistical data were conducted.

The inhibitory effect (antimutagenic activity) was calculated from the following data using the formula:

$$\text{Inhibition (\%)} \frac{(a-b)*100}{a-c}$$

where

a - number of histidine revertants induced by mutagen;

b - number of histidine revertants induced by mutagen in the presence of antimutagen;

c - number of revertants, growing in the presence of only the antimutagen.

RESULTS AND DISCUSSION

Microorganisms in the process of their life are able to produce substances, which include exopolysaccharides (EPS). Lactic acid bacteria synthesize viscous EPS into the environment (Ammor and Mayo 2007), which, in our opinion, will participate in the formation of functional and technological properties and structure formation of minced meat. In this regard, the concentration of EPS and viscosity are selected as one of the important indicators in the selection of cultures for practical use. The bioactivity of probiotic cultures was judged by the number of viable cells, indicating the state of the population of microorganisms, and the specific growth rate, characterizing the state of the cell. The results of the studies are presented in **Table 1**.

The analysis of the obtained results shows that all the presented microorganisms have high biochemical activity, as evidenced by the number of viable cells 10^{10}-10^{11} CFU/cm^3.

The combined starter culture consisting of Pediococcus pentosaseus, Staphylococcus xylosus,

Table 1. Comparative characteristics of biotechnological potential of lactic acid microorganisms

Strains	Indicators			
	Viscosity, cSt	Specific growth rate, 1 / hour	EPS concentration, KMG / ml	Number of viable cells, CFU / cm³
P, freudenreichii subsp freudenreichii AC-2500	1.88	0.39	0.65	$1*10^{11}$
P. cyclohexanicum Kusano AC-2260	3.86	0.36	1.25	$2*10^{10}$
Pediococcus pentosaseus, Staphylococcus xylosus, Staphylococcus carnosus	5.71	0.56	685	$7*10^{11}$
P. cyclohexanicum Kusano AC-2259	4.44	0.18	1.58	$5*10^{10}$
P. freudenreichii subsp shermanii AC-2503	2.23	0.54	1.25	$1*10^{10}$
Bifidum longum 339M	4.65	0.58	2.30	$3*10^{10}$
P. shermanii KM-186	7.38	0.77	10.58	$5*10^{11}$

Staphylococcus carnosus has a high specific growth rate and low viscosity. This property is important for reducing the water-binding capacity of dry sausages in order to quickly remove moisture from the product. As is known, combined starter cultures have greater biochemical activity compared to monocultures (Hugas and Monfort 1997). These data can be taken into account for meat products with low humidity.

This culture of lactic acid bacteria also has a sufficiently high specific growth rate - 0.54 h and moderate viscosity, so they can be used for the production of meat products with intermediate humidity.

The results of the research served as the basis for the development of a new approach that allow implementing the properties of lactic acid bacteria in the technology of dry sausages.

This approach is applied in further studies in the development of technology of dry sausages with lactic acid bacteria. On the basis of the obtained results, it can be stated that the studied cultures have a high biotechnological potential, and taking it into account it is possible to select microorganisms in accordance with the requirements of the technological process of production of dry sausages.

Various additives used in the food industry and chemicals in agriculture contain varying amounts of carcinogens and mutagens that adversely affect human health.

In this regard, it is of interest to reduce the mutagenic load by biotechnological processing of raw materials using probiotic microorganisms.

Lactic acid bacteria have high antimutagenic properties. It should be noted that the antimutagenic properties depend on the species and strain of lactic acid bacteria, as well as on the conditions of cultivation, the composition of nutrient media and other factors (Khorolsky and Gabaraev 1986, Lankaputhra and Shah 1998).

In this regard, the research was aimed at studying the antimutagenic activity of probiotic microorganisms and selecting the most promising strains for the production technology of dry sausages. To determine the antimutagenic activity, the Ames test was used, and the Staphylococcus xylosus test strain was used as an indicator of mutagenicity. In the first series of experiments, the antimutagenic activity of cells and culture fluids was determined.

The greatest antimutagenic effect is shown by the cells of propionic acid bacteria. Antimutagenicity (AM) of three-stem culture is 80 %, and R. shermanii KM-186-74 %.

The culture fluid of the studied strains also has antimutagenic activity, but is slightly lower than in the cells of microorganisms and is (25-55)%. According to Alekperov and Vorobieva (Vorobjeva et al. 1990) antimutagens of culture fluid are a number of amino acids - arginine, histidine, methionine, cysteine and glutamic acid, which produce lactic acid bacteria. Apparently, this is due to the antimutagenic effect.

The mechanism of the antimutagenic activity of the protein factor is not completely clear. It can be assumed that the exogenous protein (peptide) functions as a regulatory sensory protein, the oxidation of which induces a signal at any stage of the cellular regulatory cascade, as a result of which the natural AM defense is activated. In this case, the protein acts as a bioantimutagen, inhibiting the mutagen through the interaction of DNA-repair systems. Am-protection of propionic bacteria not only “stands guard” the preservation of its own genotype, but can be used to stabilize the genotypes and microbial fermentation of other organisms.

It should also be noted that the substances of the cell extract have desmutagenic properties and bind, inactivate or prevent transport of mutagens into bacterial cells. But if the mutagen does penetrate in the cell, it can be inactivated by intracellular antimutagens: amino acids of the cell pool, glutathione, enzymes of antiradical protection, and in the case of DNA damage, as we noted earlier, antimutagens reparogens come into play.

The obtained results are consistent with the literature data that propionic bacteria exhibit multilevel protection against environmental mutagens. The experiments have shown that the greatest antimutagenic effect is provided by cells of three-strain culture of propionic acid bacteria and combined starter culture of propionic acid and bifidobacteria.

When choosing starting culture for the production of meat products, their resistance to table salt is taken into account. It is known that lactic acid bacteria are resistant to sodium chloride (Belous et al. 2019, Eldefrawy et al. 2018, Samimi et al. 2019, Zinina et al. 2018) however, in the literature there is no information about the manifestation of these properties by individual strains. Therefore, the selection of the most promising strains of lactic acid bacteria for the production of sausages with a high salt content is of great interest. In this regard, the

Table 2. Effect of sodium nitrite concentration on stability of probiotic microorganisms

Strain	Number of viable cells (CFU/cm³) depending on salt concentration, %				
	Control	2	4	6	8
Pediococcus pentosaseus, Staphylococcus xylosus, Staphylococcus carnosus	$2*10^{10}$	$1*10^{10}$	$6*10^{12}$	$12*10^{10}$	$1*10^{10}$
Bifidum longum 339M	$1*10^{9}$	$5*10^{8}$	$1*10^{10}$	$2*10^{7}$	$1*10^{7}$

Table 3. Resistance of starter culture to salt and bile concentration

#	Types of lactic acid starter cultures	Salt concentration, %				Bile concentration, %		
		2	4	6	7	20	30	40
1	Pediococcus pentosaseus,	+	+	+	-	+	+	-
2	Staphylococcus xylosus,	+	+	+	-	+	+	-
3	Staphylococcus carnosus	+	+	+	+	+	+	+
4	B.longum B379	+	+	+	-	+	+	-

+ presence of turbidity
- lack of turbidity

resistance of probiotic microorganisms to different salt concentrations has been studied. For this purpose different concentrations of table salt were added to the nutrient media during the cultivation of microorganisms - 2, 4, 6, 8 %.

The most characteristic and adequate indicator of the state of stress is a decrease in the growth rate and viability of cells of probiotic microorganisms. The biochemical activity of probiotic cultures in different salinity conditions was determined by these indicators.

Analysis of the results presented in **Tables 2** and **3** shows that with an increase in the salt concentration to 4 %, the number of viable cells remains at a sufficiently high level and is 10^{10}-10^{11} CFU/cm^3. Further increase of salt concentration leads to decrease of viability of cells of probiotic microorganisms.

Cultures of lactic acid bacteria and combined lactic acid-based starter cultures show the highest resistance to extreme salinity. At a salt concentration (6-8) %, the number of viable cells in these cultures is 10^{10} CFU/cm^3.

As experiments have shown, while the concentration of sodium nitrite increases, the growth of all strains slows down. It was found that at a concentration of sodium nitrite of 0.10 mg/ml, all studied strains exhibit high resistance and the number of viable cells is 10^{10} - 10^{11} CFU/ml. Increasing the concentration of sodium nitrite to 0.20 mg / ml slightly reduces their resistance. The number of cells of probiotic microorganisms is 10^{10}-10^{11} CFU / ml (**Table 2**).

Table 4. Effect of sodium nitrite to antimutagenic activity of lactic acid bacteria

Strain	Sodium nitrite concentration, mg/ml	Average number of revertants on cup	Inhibition, %
Pediococcus pentosaseus, Staphylococcus xylosus, Staphylococcus carnosus	control	2987	32.0
	0.1	2874	31.6
	0.14	3145	35.2
	0.2	3265	36.8
Bifidum longum 339M	control	3334	27.5
	0.1	3654	31.9
	0.14	3028	32.5
	0.2	2956	28.3

The greatest resistance to high concentrations of sodium nitrite has a three-strain starter. For guaranteed color formation in sausage technology, the amount of sodium nitrite added is 10 g per 100 kg of unsalted raw materials. As it can be seen from the results of the experiment, all the studied cultures are resistant to the normative dose of sodium nitrite.

As follows from the data of **Table 4** lactic acid bacteria in the presence of sodium nitrite accumulate antimutagens. It should be noted that with an increase in the dose of sodium nitrite, there is a higher antimutagenic activity of lactic acid bacteria, which indicates the induction of antimutagenesis.

CONCLUSION

The possibility of induction of antimutagenesis shows that the antimutagenic activity of bacteria is not limited to their sorption properties. The mechanism of induction can be associated with the activation of enzymes of superoxide dismutase and catalase that protect propionic bacteria from oxidative stress - nitroxyl radical formed during the nitrosation reaction. The revealed regularities allow not only understanding the principles of metabolic organization in lactic acid bacteria, but also serve as a scientific justification for reducing the toxicity of sodium nitrite. Thus, the data obtained by us on the ability of probiotic microorganisms to synthesize antimutagenic substances create real prerequisites for the creation of new biotechnologies of meat products with antimutagenic properties.

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