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## STUDY OF LACTIC ACID BACTERIA AS A BIO-PROTECTIVE CULTURE FOR MEAT

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**Summary.** Loss prevention and food quality maintenance are primarily associated with protection against the negative impact of microorganisms and their metabolites during manufacture and storage. In this regard, in recent years, the issue of the goods safety is of the top-priority in the food production. Meat and meat products are the most labour-intensive and expensive to manufacture. Their main components (protein, fat, etc.) are a favourable environment for development of a variety of microorganisms.

This paper presents the results of the biotechnological property research of the *Lactobacillus* genus collection strains, their effect on the microorganisms directly isolated from meat and on the collection strains (saprophytic, conditionally pathogenic and pathogenic microorganisms). In particular, the antagonistic activity regarding the indicator and collection microorganisms, acid activity and ability to survive at high salt concentrations and low above-zero temperatures have been studied. Based on the experimental results, the most active strains for further study and use in the meat industry have been identified.

**Key words:** microbiota of meat, *Lactobacillus*, storage life, antagonistic action.

## ДОСЛІДЖЕННЯ МОЛОЧНОКИСЛИХ БАКТЕРІЙ ЯК БІОЗАХИСНОГО ФАКТОРУ М'ЯСА

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**Анотація.** У роботі наведено результати досліджень впливу колекційних штамів бактерій роду *Lactobacillus* на мікроорганізми, які виділені безпосередньо з м'яса та на колекційні штами (сапрофітні, умовнопатогенні та патогенні мікроорганізми). Зокрема, вивчено антагоністичну активність щодо індикаторних та колекційних мікроорганізмів, активність кислотоутворення, здатність до виживання при високих концентраціях солі і при низьких плюсових температурах. За результатами дослідів виявлено найбільш активні штами для подальшого вивчення та використання у м'ясній промисловості.

**Ключові слова:** мікробіота м'яса, *Lactobacillus*, строк зберігання, антагоністична дія.



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### Introduction

Continuous product quality and safety assurance is a primary objective for the meat industry experts. Fresh meat has a very limited storage life creating difficulties for the manufacturers and being a potential threat to the consumers. In this context, sales of the meat products are geographically limited to the production location, and transportation and storage require special conditions.

Meat is a favourable nutrient substrate for the microorganisms, where they find everything necessary for them – the sources of carbon, nitrogen, vitamins and minerals. The moisture content and pH of meat also contribute to their development, therefore meat goes off quickly. In the inner meat layers of a healthy

animal no microorganisms or rare cells of them are found directly after slaughter. When processing a carcass, contamination of its surface with the microorganisms, which later can cause damage to the product, takes place.

The microbiota of the meat surface is varied and depends on many factors: animal skin purity before slaughter, slaughter conditions and initial carcass processing (the method of skinning), cleanliness of tools, air contamination. As a result, the number of microorganisms per 1 cm<sup>2</sup> of the meat surface area can widely vary (10<sup>2</sup>-10<sup>6</sup> mu and more).

The composition of the meat microbiota is varied. These are mainly aerobic and optionally anaerobic vegetative gram-negative rods, including *Escherichia coli*, *Proteus* and lactic acid cocci. There are aerobic

and anaerobic spore-forming bacteria, yeast and mold found less in quantity. Meat can be contaminated with the pathogenic bacteria, such as clostridia or salmonella. In this regard, the ways to protect meat from spoiling are searched for [1]. Here, the use of the lactic acid bacteria, producing a number of the biologically active substances preventing the pathogenic microbiota growth and reproduction and acting as a natural preservative is quite promising.

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#### Problem formulation

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Production of the meat products has always been a time-consuming and costly industry. Given that the meat product surface is contaminated with various microorganisms, one can say that just these microorganisms degrade the organoleptic properties of the products, cause changes in the protein and fat composition and produce toxic substances. Therefore, the industrial production places a premium on the meat and meat products' quality maintenance and storage life extension, and development of new methods and technologies to extend the storage life of the meat products is of special scientific and practical interest [2].

The goals and objectives of the study:

- to obtain and identify the meat surface microbiota members;
- to study the antagonistic properties of different strains of the collection lactic microorganisms (relative to the meat surface microbiota and collection saprophytic, conditionally pathogenic and pathogenic microorganisms);
- to study the halo- and thermo resistance of the collection strains of the lactic acid bacteria.

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#### Literature review

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Currently, the demand for the chilled meat and meat products made of it, including the semi-finished and ready-to-eat products is increasing. These products are perishable. So, in the process of their production, storage, transportation and sales it is necessary to create the appropriate conditions to prevent the development and proliferation of the microorganisms being a major factor in spoilage of the meat products and risk to the consumers' health [2].

The pathogenic and conditionally pathogenic microorganisms such as *Salmonella*, *Listeria*, *Yersinia*, *Campylobacter*, *Clostridium* and *Escherichia* are especially dangerous for health. In practice, the term of the meat products life is determined by the storage period until the signs of organoleptic changes appear, and depends mainly on the initial microorganism content in the raw material, type of the meat product and packaging, as well as the storage temperature [1-2]. The biotechnological processes using the beneficent microorganisms, the lactic acid ones, first of all, as well as enzymes, are already widely used in the food industry on the contemporary technical level.

The lactic acid bacteria are used in a number of the food industry sectors. Due to formation of the large lactic acid amounts, to which they are largely tolerant, the lactic acid bacteria under the appropriate conditions can quickly generate, replacing other microorganisms. Due to this, they are easy to cultivate on the selective media and easy to obtain. In the meat industry, the lactic acid bacteria are important for the fermented sausages manufacture because they positively influence the texture and viscosity of the sausage stuffing [3].

Action of the lactic acid microorganisms – *Lactococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc* – resulting in the inhibition of undesirable and formation of certain microbiota, is the antibacterial substances synthesis, such as organic acids, carbon dioxide, hydrogen peroxide, diacetyl and bacteriocins. This explains their complex antimicrobial action, which allows using them as a natural food preservative [1].

Application of the selected strains opens prospects for deriving products with the new valuable qualities (original flavour, aroma, appropriate colour, texture). It makes sense for the strains received to be able to actively suppress the harmful microbiota developing on the surface and in the bulk of the meat products [4].

In this regard, the information about the microbiological status change in the meat raw material and products is of special interest. The information on-top available domestically is extremely limited.

**Purpose of the study.** Studying the meat surface microbiota and selecting the most effective lactic acid bacteria strains to inhibit it.

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#### Study of lactic acid bacteria as a bio-protective culture for meat

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The muscle tissue of the healthy animals theoretically should be sterile. However, when animals are slaughtered at the slaughterhouses, meat normally contains a different number of microorganisms. It should be noted that under various environmental problems, changes in the animal feeding and other factors, there are quantitative and qualitative microorganism composition changes forming their resistance to the unfavourable factors [5]. Therefore, in this work, in addition to the collection microorganisms, the bacteria isolated from meat have been studied.

According to the current regulations, the following microbiological indicators are governed in the meat industry: the number of mesophilic aerobic and optionally anaerobic microorganisms, presence of *Escherichia coli* bacteria (representatives of *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Serratia* genus), and opportunistic pathogens (bacteria of *Proteus*, *Bacillus cereus* and *Clostridium rferfringens* genus) [7,8].

In the fresh meat, the representatives of *Aeromonas*, *Brochothrix*, *Clostridium*, *Carnobacterium*, *Lactobacillus* and *Leuconostoc* genus can be found, as

well as *Enterobacteriaceae* and *Micrococcaceae* genus microorganisms. Often, most of the contaminants belong to *Pseudomonas* genus and related genera. In addition, yeasts (*Cryptococcus*, *Candida*, *Rhodotorula*, *Torulopsis*) and mold can be present in the fresh meat [6].

For the microbiological assessment of the raw meat quality, the flank, neck and ham samples were taken since these carcass parts are often exposed to the microbial contamination. The number of mesophilic aerobic and optionally anaerobic microorganisms was calculated by inoculation of tenfold dilutions of an average sample washing using the meat infusion agar and by incubation at 37 °C for 24 – 48 hours.

For generic assignment, 10 cultures were selected to study their basic biological properties (cultural, morphological, physiological and biochemical) in accordance with Bergey's Manual of Determinative Bacteriology. The study pursued allowed to designate these cultures as: *Bacillus* (*Bacillus* sp. 1, *Bacillus* sp. 2, *Bacillus* sp. 3), *Kurthia* (*Kurthia* sp.), *Planococcus* (*Planococcus* sp. 1, *Planococcus* sp. 2), *Micrococcus* (*Micrococcus* sp. 1, *Micrococcus* sp. 2), *Sarcina* (*Sarcina* sp.) and *Staphylococcus* (*Staphylococcus* sp.).

To inhibit or slow the development of microorganisms, various traditional methods (cold, high temperature, antiseptics, etc.) and biologically active substances, enzymes and metabolites of microorganisms, including the lactic acid ones, are used [1,8].

One of the promising methods is using the lactic acid microorganisms. So, the next step was to test the antagonistic action of the collection lactic acid bacteria, namely *Lactobacillus* genus, toward the indicator microorganisms directly isolated from meat, and the collection strains of bacteria.

Eight strains of *Lactobacillus* genus bacteria were selected from the microorganisms collection of Odessa I.I. Mechnikov National University: *L. acidophilus* 147, *L. casei* s/sp. *Rhamnosus* 283, *L. casei* s/sp. *tolerans* 187, 290, *L. delbrueckii* s/sp. *Lactis* 013, *L. gasseri* 149, *L. plantarum* 12, 1005. The collection strains of the lactic acid bacteria were previously incubated in the liquid MRS medium at 37 °C for 48 – 72 hours. The antagonistic activity of the lactic acid bacilli was determined by the volume displacement method.

All the lactic acid bacteria strains studied proved to be active antagonists towards the microorganisms isolated from the flank, neck, ham and collection strains (table 1,2).

**Table 1 – Antagonistic activity of the lactic acid bacteria studied toward the microorganisms isolated from the meat surface**

Lactic acid microorganisms	Diameter of delay and lack of growth of the microorganisms isolated from meat, mm									
	1	2	3	4	5	6	7	8	9	10
<i>L. gasseri</i> 149	25^	20*	22*	27*	25*	34*	24^	24*	25^	29^
<i>L. casei</i> s/sp. <i>rhamnosus</i> 283	21^	21*	23*	26*	24*	25*	25^	26*	31^	25*
<i>L. casei</i> s/sp. <i>tolerans</i> 290	25^	20*	28*	32*	20*	28*	28^	23*	45*	41*
<i>L. acidophilus</i> 147	14^	20^	22^	28^	33*	32*	20^	21*	-	24^
<i>L. casei</i> s/sp. <i>tolerans</i> 187	27^	25^	20*	23*	26^	31*	27*	23*	24*	28*
<i>L. delbrueckii</i> s/sp. <i>lactis</i> 013	25^	28^	29*	30*	24*	33*	28*	31*	30*	40*
<i>L. plantarum</i> 1005	18^	17*	-	12*	27^	22*	21^	19*	-	-
<i>L. plantarum</i> 12	25^	26^	23*	26*	25*	35*	27^	24*	23*	30*

Note: “-” no effect, “\*” no growth, “^” stasis. 1–*Bacillus* sp.1; 2–*Bacillus* sp.2; 3–*Bacillus* sp.3; 4–*Kurthia* sp.; 5–*Planococcus* sp.1; 6–*Planococcus* sp.2; 7–*Micrococcus* sp.1; 8–*Micrococcus* sp.2; 9–*Sarcina* sp.; 10–*Staphylococcus* sp.

**Table 2 – Antagonistic activity of the lactic acid bacteria studied toward the collection microorganism strains**

Lactic acid microorganisms	Diameter of delay and lack of growth of the collection microorganisms, mm								
	1	2	3	4	5	6	7	8	9
<i>L. gasseri</i> 149	31^	25^	25^	20*	25^	20^	25*	19^	25^
<i>L. casei</i> s/sp. <i>rhamnosus</i> 283	35*	25*	25*	23^	33*	22^	28*	19^	32^
<i>L. casei</i> s/sp. <i>tolerans</i> 290	44*	26*	34*	24^	25^	24^	28*	21^	34^
<i>L. acidophilus</i> 147	30*	21^	-	19^	21*	18^	21*	-	21^
<i>L. casei</i> s/sp. <i>tolerans</i> 187	29*	25*	-	20*	30^	22^	23*	-	22^
<i>L. delbrueckii</i> s/sp. <i>lactis</i> 013	29*	27*	-	23*	40*	22^	24*	-	24^
<i>L. plantarum</i> 1005	33*	22^	30*	22^	28^	17*	20*	18^	18^
<i>L. plantarum</i> 12	27*	35*	40*	25*	30^	23^	29*	20^	26^

Note: “-” no effect, “\*” no growth, “^” stasis. 1–*Planococcus citreus*; 2–*Escherichia coli*; 3–*Micrococcus luteus*; 4–*Staphylococcus aureus*; 5–*Bacillus megaterium*; 6–*Bacillus subtilis*; 7–*Salmonella enteritidis*; 8–*Proteus vulgaris*; 9–*Bacillus cereus*.

As it is seen from the results listed in Tables 1 and 2, the lactic acid bacteria strains inhibit growth of the indicator bacteria both, isolated from meat and the collection ones. Herewith, the diameter of the “no growth” area of the bacteria isolated ranged from 12±1.1 mm to 41±1.1 mm. For the collection strains, this index was 17±1.1 mm to 44±1.1 mm, respectively. The most effective antagonists of the microbes contaminating the raw meat appeared to be *L. delbrueckii s/sp. Lactis* 013, *L. plantarum* 12 and *L. casei s/sp. tolerans* 290.

As you know, the antagonistic activity of the lactic acid bacteria is most commonly associated with the ability to produce organic acids. Therefore it was appropriate to investigate the acid formation activity of the lactic acid bacilli under study.

The maximum acidity was determined by titration. The daily culture of *Lactobacillus* liquid in MRS-broth enriched with 5 % glucose was titrated with 0.1 n NaOH with phenolphthalein indicator. Sterile environment served as control. The experiment data are shown in Table 3.

**Table 3 – Acid formation activity of the lactic acid bacteria strains studied**

Strain	T
<i>L. gasseri</i> 149	123.4
<i>L. casei s/sp. rhamnosus</i> 283	145.3
<i>L. casei s/sp. tolerans</i> 290	166.8
<i>L. acidophilus</i> 147	154.1
<i>L. casei s/sp. tolerans</i> 187	137.6
<i>L. delbrueckii s/sp. lactis</i> 013	152.1
<i>L. plantarum</i> 1005	157.1
<i>L. plantarum</i> 12	169.6

According to the results presented in Table 3, it is obvious that the lactic acid bacteria produce significant amounts of organic acids, thus creating unfavourable conditions for the development of other microorganisms, particularly putrid ones being the main agents of the microbial spoilage. The acid formed by the bacteria of *Lactobacillus* genus can be considered as a natural preservative which can be used to extend the storage life of meat.

To identify the impact of salt contained in the meat products, the lactic acid bacteria were tested for their salt resistance.

For this purpose, the liquid MRS medium containing NaCl in the concentrations of 2.5; 5.0; 7.5 and 10.0 % was prepared. The daily cultures of lactic acid bacteria were used for the preparation of suspensions in the concentration of 10<sup>9</sup> CFU/cm<sup>3</sup>. 0.1 cm<sup>3</sup> of each suspension was introduced to the liquid medium containing NaCl in the appropriate concentrations. Visual analysis of the results was made within 2 weeks of incubation at 37 °C based on the studied cultures' growth rate. The obtained data are presented in Table 4.

**Table 4 – Growth rate of the lactic acid bacteria under different NaCl concentrations**

Strain	MRS-broth			
	2.5 %	5.0 %	7.5 %	10.0 %
<i>L. gasseri</i> 149	++++	++++	++++	+
<i>L. casei s/sp. rhamnosus</i> 283	++++	++++	++++	+
<i>L. casei s/sp. tolerans</i> 290	++++	++++	++++	++
<i>L. acidophilus</i> 147	++++	++++	++++	++
<i>L. casei s/sp. tolerans</i> 187	++++	++++	++++	+
<i>L. delbrueckii s/sp. lactis</i> 013	++++	++++	+++	++
<i>L. plantarum</i> 1005	++++	++++	++++	+++
<i>L. plantarum</i> 12	++++	++++	++++	+++

Note: “++++” - exceptional growth rate, “+++” - high, “++” - medium, “+” - low, “±” - very low, “-” - no growth.

According to the results presented in Table 5, it is obvious that bacteria of *Lactobacillus* genus show high rate of growth in the media with NaCl concentrations from 2.5 % to 7.5 %. Such lactic acid bacteria strains as: *L. acidophilus* 147, *L. delbrueckii s/sp. lactis* 013 and *L. plantarum* 12 showed the largest biomass accumulation with salt.

When studying the biotechnological properties of *Lactobacillus* genus bacteria, their ability to develop at different temperatures (5, 10, 15, 20, 25 °C) in the same liquid medium was investigated. The growth rate of the cultures studied has been evaluated for 2 weeks. The results are shown in Table 5.

**Table 5 – Growth rate of the lactic acid bacteria at different temperatures**

Strain	MRS-broth				
	5° C	10° C	15° C	20° C	25° C
<i>L. gasseri</i> 149	+	+++	++++	++++	++++
<i>L. casei s/sp. rhamnosus</i> 283	++	+++	++++	++++	++++
<i>L. casei s/sp. tolerans</i> 290	+++	++++	++++	++++	++++
<i>L. acidophilus</i> 147	+++	++++	++++	++++	++++
<i>L. casei s/sp. tolerans</i> 187	+++	++++	++++	++++	++++
<i>L. delbrueckii s/sp. lactis</i> 013	+++	+++	++++	++++	++++
<i>L. plantarum</i> 1005	++	++++	++++	++++	++++
<i>L. plantarum</i> 12	+++	++++	++++	++++	++++

Note: “++++” - exceptional growth rate, “+++” - high, “++” - medium, “+” - low.

The results of the study presented in Table 5, indicate that the most favourable temperature for growth of the lactic acid bacteria strains ranges from 10° C to 25° C. But

there were strains which showed a high capacity for survival even at 5° C: *L. acidophilus* 147, *L. delbrueckii s/sp. lactis* 013 and *L. plantarum* 12.

### Conclusion

The antagonistic action research shows that the lactic acid bacteria strains under study have a high antagonistic action towards the indicator microorganisms isolated from meat, as well as saprophytic, conditionally pathogenic and pathogenic bacteria. The data on the acid-forming ability show that the lactic acid bacteria produce a significant amount of acid contributing to suppression of other microbiota.

The experiments on the lactic acid bacteria salt resistance and growth rate at low above-zero temperatures

show that strains of bacteria of *Lactobacillus* genus have high rate of growth in the media with NaCl concentrations from 2.5 % to 7.5 % and develop well at low above-zero temperatures. The most favourable temperature is between 10 °C and 25 °C, but there were strains of the lactic acid bacteria which develop at 5 °C.

The obtained results indicate that bacteria of *Lactobacillus* genus show the expediency of their further research for using in the meat industry to increase the storage life and inhibit the undesirable microbiota.

Also, the obtained results provide a basis for further research in order to create a complex microbial formulation to protect meat and meat products from the microbial spoilage.

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## ИССЛЕДОВАНИЕ МОЛОЧНОКИСЛЫХ БАКТЕРИЙ КАК БИОЗАЩИТНОГО ФАКТОРА МЯСА

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**Аннотация.** В данной работе представлены результаты исследований свойств коллекционных штаммов бактерий рода *Lactobacillus*, их влияние на микроорганизмы, которые выделены непосредственно из мяса и на коллекционные штаммы (сапрофитные, условно патогенные и патогенные микроорганизмы). В частности, изучено антагонистическую активность, на индикаторные и коллекционные микроорганизмы, активность кислотообразования, способность к выживанию при высоких концентрациях соли и при низких плюсовых температурах. По результатам опытов выявлены наиболее активные штаммы, для дальнейшего изучения и использования в мясной промышленности.

**Ключевые слова:** микробиота мяса, *Lactobacillus*, срок хранения, антагонистическое действие

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