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Astana International University, Republic of Kazakhstan, Astana

ORCID: <https://orcid.org/0000-0001-7862-7637>

*e-mail: adamzhanova@mail.ru

SCIENTIFIC FOUNDATIONS FOR DEVELOPING FUNCTIONAL DAIRY PRODUCT STARTER CULTURES IN PAVLODAR REGION

The article examines the study of microorganisms isolated from traditional fermented dairy products with the aim of developing starter cultures for the production of functional dairy products specific to the Pavlodar region. The research focused on the physiological and biochemical properties, antibiotic resistance, antagonistic activity, and biocompatibility of lactic acid bacteria, including Lactobacillus gallinarum, Streptococcus thermophilus, Lactococcus lactis subsp. lactis, and Lactobacillus fermentum. These strains were selected for further study due to their high metabolic activity, ability to efficiently produce lactic acid, antagonistic activity against pathogenic microflora, and resistance to unfavorable production conditions.

The methodology involved the isolation of pure cultures using various nutrient media, biochemical analyses, and the evaluation of the technological potential of the isolated strains. The selected strains demonstrate potential for the creation of direct-inoculation starter cultures capable of improving the quality and safety of fermented products, as well as meeting the growing demand for functional food products.

The article discusses the prospects of utilizing these starter cultures to produce probiotic-rich products, such as yogurt, kefir, and others, with high nutritional value. The results lay the groundwork for the industrial implementation of technologies aimed at producing high-quality fermented dairy products that support consumer health and contribute to the development of the regional dairy industry.

Keywords: milk, fermentation of milk, microorganisms, lactobacilli, mesophilic lactic acid streptococci, thermophilic lactic acid streptococci, acidophilic lactic acid sticks.

Introduction

Sourdough cultures are pure cultures or mixtures of lactic acid bacteria used in biotechnological processes to initiate milk fermentation. Starter cultures can be liquid, frozen, or dry. Dry starter cultures are freeze-dried biomass containing one or more types of microorganisms, each performing a specific role in the fermentation process [1]. Starter cultures can be traditional, used for products like yogurt, kefir, ryazhenka, sour cream, cottage cheese, and matsoni, or probiotic, designed to produce functional products with a high concentration of beneficial probiotic microorganisms [2].

Objective of the Study: To develop starter cultures for the production of fermented dairy products specific to the Pavlodar region, based on scientific and practical foundations.

To achieve this goal, the following interrelated research tasks are proposed:

Study the composition and properties of traditional dairy products produced in the Pavlodar region.

Investigate the processes of obtaining, as well as the composition and properties of the microflora of traditional fermented dairy products, along with the technological aspects and prospects for production development in the region.

Develop an organizational research scheme and determine experimental objects and materials.

Analyze the cultural, morphological, physiological, and biochemical properties, antibiotic resistance, antagonistic activity, and biocompatibility of microorganisms isolated from traditional dairy products.

Develop a symbiotic starter culture based on lactobacilli isolated from local fermented dairy products to enhance fermentation quality.

Conduct market research and develop a strategy for product promotion and pricing for fermented dairy products.

Create a business plan for establishing a laboratory and perform economic calculations for sourdough culture production [3].

Materials and Methods

Microorganisms and Sources

This research primarily focused on microorganisms isolated from traditional fermented milk beverages. The studied microorganisms included a wide variety of lactic acid bacteria, such as *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* biovar *diacetilactis*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, and *Lactobacillus gallinarum* [4]. These microorganisms were chosen for their historical importance and industrial relevance in fermented dairy product production.

Isolation of Pure Cultures

The isolation process for pure cultures of lactic acid bacteria was conducted in several well-defined stages to ensure the reliability and reproducibility of results. These stages included:

1 Selection of Sources. Traditional fermented milk beverages and spontaneously fermented dairy products from various regions were carefully selected as sources of lactic acid bacteria. Additionally, intestinal contents from calves and infants were used as sources for isolating *Lactobacillus acidophilus*.

2 Sample Collection and Preparation. Samples were collected in sterile containers under aseptic conditions to avoid contamination. Each sample was labeled with specific information, including the source, location, and collection date.

3 Enrichment of Lactic Acid Microflora. The samples were inoculated into a liquid nutrient medium specifically designed to favor the growth of lactic acid bacteria. This enrichment step allowed the selective proliferation of the target microorganisms.

4 Isolation of Pure Cultures. The enriched cultures were streaked onto solid nutrient media, such as MRS agar, to isolate individual colonies. Distinct colonies were selected based on morphological characteristics and transferred to fresh solid media to obtain pure cultures.

5 Reinoculation in Sterile Milk. Pure cultures were reinoculated into sterile milk to confirm their ability to grow and produce lactic acid. This step also allowed the evaluation of their fermentation capabilities and their potential application in dairy product production.

6 Characterization of Biological Properties. The isolated strains were subjected to comprehensive biochemical and physiological tests to characterize their metabolic activities, acid tolerance, enzymatic properties, and other biological traits. These properties were analyzed to determine their potential for industrial applications, such as probiotic formulations or starter culture development.

Regional Variations in Microbial Sources

Thermophilic lactic acid streptococci and *Lactobacillus delbrueckii* subsp. *bulgaricus* were predominantly isolated from spontaneously fermented dairy products originating from southern regions, which are known for their traditional fermentation practices. In contrast, *Lactobacillus acidophilus* was isolated from the gastrointestinal tract of calves and infants, highlighting its adaptability to diverse environments [5].

Materials, Reagents, and Reference Strains

A wide range of materials and reagents were employed throughout the study. The research utilized several reference strains for comparative analysis, including:

- *Escherichia coli* B-6954
- *Bacillus fastidiosus* B-5651
- *Pseudomonas fluorescens* B-3502
- *Pseudomonas aeruginosa* ATCC 9027
- *Leuconostoc mesenteroides* B-8404
- *Candida albicans* ATCC 885-653
- *Staphylococcus aureus* ATCC 25923

Additionally, specific sugars (e.g., lactose, glucose, and sucrose) and chemical reagents were used for metabolic characterization and growth studies.

Analytical Techniques

To assess the metabolic and enzymatic properties of the isolates, various analytical techniques were employed. These included:

- pH measurement and titratable acidity for fermentation profiling.
- Gas chromatography for volatile compound analysis.
- Enzymatic assays for determining proteolytic and lipolytic activity.
- Molecular identification using 16S rRNA gene sequencing for precise taxonomic placement of the strains.

This comprehensive approach ensured a detailed understanding of the isolated strains, enabling their evaluation for potential industrial applications. The study also provided insights into the microbiota of traditional fermented dairy products and their contribution to product quality and flavor.

Results and Discussion

To isolate mesophilic lactic acid streptococci, 1 g of sample was ground in a sterile mortar and diluted 1:10 in saline solution. This suspension was inoculated in sterile milk (10 cm³ in 0.25–0.5 cm³ aliquots). For cultures derived from fermented milk products, one drop of the product was inoculated directly into sterile milk. Incubation was conducted at 25–30 °C until coagulation occurred [1].

Isolation of thermophilic lactic acid bacteria followed a similar protocol, with cultures incubated at 40–43 °C for streptococci and *Lactobacillus bulgaricus* and at 37 °C for *Lactobacillus acidophilus*, with a 48-hour incubation period [7].

Strains intended for production applications must meet specific criteria: thermophilic streptococci should not grow in milk containing penicillin (0.01 IU/cm³) and should thrive in hydrolyzed milk with up to 2 % NaCl and 0.1% methylene blue. Strains of *Lactobacillus acidophilus* must demonstrate resistance to 0.4 % phenol, 20 % bile, and a medium pH of 8.3, with significant antibiotic activity against spoilage flora, *Staphylococcus*, *Proteus*, and *Escherichia coli*. Strains of *Lactobacillus bulgaricus* should produce acetaldehyde, a desirable flavor compound.

Selected production-worthy lactic acid bacteria strains may be stored frozen at minus 18 to minus 25 °C for 4–6 months or in a freeze-dried state in sealed

ampoules. In this form, they can be stored at 3–5 °C or minus 18 to minus 25 °C for up to ten years or more. Each concentrate must contain 150–300 billion cells per gram with a moisture content not exceeding 3.5 %, and the presence of extraneous, non-pathogenic microflora should not exceed 10 cells per gram [8].

The isolation of microorganisms from traditional fermented milk beverages such as ayran and koumiss was performed by inoculating 1 ml of the beverage in nutrient media, followed by incubation at 37 °C in a CO₂-enriched environment for 1–5 days. Primary isolation was achieved using a variety of media, including skimmed milk, MRS broth, heart-brain infusion, milk agar with 3 % agar, fish-peptone agar, and MRS agar [9].

Conclusion

The conducted studies of the physiological and biochemical properties, antibiotic resistance, antagonistic activity, and biocompatibility of microorganisms isolated from national fermented milk beverages have provided a solid foundation for selecting promising strains of lactic acid bacteria. The research highlighted the unique characteristics and potential applications of these microorganisms, which were carefully evaluated for their suitability in the dairy industry.

Among the tested strains, *Lactobacillus gallinarum*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis*, and *Lactobacillus fermentum* demonstrated exceptional properties that make them suitable candidates for further study and application. These strains were chosen based on their high metabolic activity, ability to produce lactic acid efficiently, and capacity to survive and function under industrial fermentation conditions. Additionally, they exhibited strong resistance to antibiotics, which is crucial for maintaining the safety and stability of fermented products in diverse environments.

The selected strains also displayed notable antagonistic activity, effectively inhibiting the growth of pathogenic and spoilage microorganisms. This characteristic is particularly important for enhancing the safety and shelf life of dairy products, making these strains valuable for producing high-quality fermented milk beverages. Furthermore, the biocompatibility of these microorganisms ensures their safe interaction with other starter cultures and their ability to integrate seamlessly into existing production processes.

The potential applications of *Lactobacillus gallinarum*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis*, and *Lactobacillus fermentum* extend beyond traditional fermentation practices. These strains hold promise for the development of functional food products enriched with probiotics, which are known for their health benefits, including improving gut microbiota balance, enhancing immune response, and preventing gastrointestinal disorders. Their utilization could contribute to the creation of dairy products with added

value, catering to the growing consumer demand for nutritious and health-promoting foods.

Investigations into their genetic and molecular profiles may further enhance our understanding of their functional capabilities and allow for targeted improvements. Ultimately, the use of these strains in direct application starter cultures can significantly improve the quality, safety, and health benefits of fermented dairy products, paving the way for innovative advancements in the dairy industry [10].

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*Ж. А. Адамжанова

Астана халықаралық университеті, Қазақстан Республикасы, Астана к.

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ПАВЛОДАР ӨҢІРІНДЕ ФУНКЦИОНАЛДЫ СҮТ ӨНІМДЕРИНЕ АРНАЛҒАН АШЫТҚЫЛАРДЫ ӘЗІРЛЕУДІҢ ҒЫЛЫМИ НЕГІЗДЕРІ

Мақалада Павлодар өңіріне тән функционалды сүт өнімдерін өндіруге арналған ашытқы мәдениеттерін өзірлеу маңсатында дәстүрлі ферменттелген сүт өнімдерінен болініп алынған микроорганизмдерді зерттеу нәтижелері қарастырылады. Зерттеу *Lactobacillus gallinarum*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis* және *Lactobacillus fermentum* сияқты сүтқышқылды бактериялардың физиологиялық және биохимиялық қасиеттеріне, антибиотиктерге тозімділігіне, антагонистік белсенделілігіне және биосыйысымдылығына негізделді. Бұл штаммдар жыгары метаболикалық белсенделілігі, сүт қышқылын тиімді өндіру қабілеті, патогендік микрофлорага қарсы антагонистік белсенделілігі және өндістемік жасадайларға тозімділігі себепті таңдалып алынды.

Әдістеме әртүрлі қоректік орталарды қолдану арқылы таза дақылдарды боліп алу, биохимиялық талдаулар жүргізу және алынған штаммдардың технологиялық өлеуеттін бағалауды қамтыды. Таңдалған штаммдар ферменттелген өнімдердің сапасы мен қауіпсіздігін арттыруға, сондай-ақ функционалды тағам өнімдеріне деген сұранысты қанагаттандыруға қабілетті тікелей енгізілеттін ашытқыларды жасасуға өлеуетті.

Мақалада осы ашытқыларды жыгары тағамдық құндылығы бар йогурт, айран және басқа да пробиотиктерге бай өнімдерді өндіруде пайдалану перспективалары талқыланады. Зерттеу нәтижелері сапалы ферменттелген сүт өнімдерін өндіруге және аймақтық сүт оперкесінің дамытуға бағытталған технологияларды оперкесінке енгізуге негіз бола алады.

Кілтті сөздер: сүт, сүтті ашыту, микроорганизмдер, лактобациллалар, мезофильді сүт қышқылы стрептококктары, термофильді сүт қышқылы стрептококктары, ацидофильді сүт қышқылы таяқшалары.

*Ж. А. Адамжанова

Международный университет Астаны, Республика Казахстан, г. Астана.

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НАУЧНЫЕ ОСНОВЫ РАЗРАБОТКИ ЗАКВАСОЧНЫХ КУЛЬТУР ДЛЯ ФУНКЦИОНАЛЬНЫХ МОЛОЧНЫХ ПРОДУКТОВ В ПАВЛОДАРСКОМ РЕГИОНЕ

*В статье рассматриваются исследования микроорганизмов, выделенных из традиционных ферментированных молочных продуктов, с целью разработки заквасочных культур для производства функциональных кисломолочных продуктов в Павлодарском регионе. В работе изучены физиологические и биохимические свойства, устойчивость к антибиотикам, антагонистическая активность и биосовместимость молочнокислых бактерий, таких как *Lactobacillus gallinarum*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis* и *Lactobacillus fermentum*. Эти штаммы были выбраны для дальнейшего изучения благодаря их высокой метаболической активности, способности эффективно продуцировать молочную кислоту, антагонистической активности в отношении патогенной микрофлоры и устойчивости к неблагоприятным условиям производства.*

Методология включала выделение чистых культур с использованием различных питательных сред, проведение биохимических анализов, а также оценку технологических перспектив использования выделенных штаммов. Были выявлены штаммы, обладающие потенциалом для создания заквасок прямого внесения, способных улучшить качество и безопасность ферментированных продуктов, а также удовлетворить растущий спрос на функциональные пищевые продукты.

В статье обсуждаются перспективы применения данных заквасочных культур для создания продуктов с пробиотическими свойствами, таких как йогурты, кефир и другие, обладающие высокой

питательной ценностью. Результаты исследований закладывают основу для промышленного внедрения технологий по производству качественных кисломолочных продуктов, способных поддерживать здоровье потребителей и способствовать развитию региональной молочной промышленности.

Ключевые слова: молоко, ферментация молока, микроорганизмы, лактобациллы, мезофильные молочнокислые стрептококки, термофильные молочнокислые стрептококки, ацидофильные молочнокислые палочки.

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Торайғыров университеті

Павлодар мемлекеттік университеті

140008, Павлодар к., Ломов к., 64, 137 каб.

«Toraighyrov University» баспасы

Торайғыров университеті

140008, Павлодар к., Ломов к., 64, 137 каб.

8 (7182) 67-36-69

e-mail: kereku@tou.edu.kz

www.vestnik-cb.tou.edu.kz