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Selection of Strains and Evaluation of Environmental Parameters in Yogurt Production Using Indigenous Bacteria

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Abstract The aim of this study was to develop a yogurt starter culture based on indigenous lactic acid bacteria (LAB) isolated in Uzbekistan and to optimize its technological parameters. The study utilized the strains Streptococcus thermophilus AWL, Lactobacillus delbrueckii subsp. bulgaricus AWL-D, and Lactobacillus acidophilus AWL. Fermentation experiments were conducted at temperatures of 37 ℃ and 42 ℃ using different starter concentrations and with the addition of a stabilizer (sodium tripolyphosphate, STPP). A comprehensive analysis included fermentation kinetics, titratable acidity (°T), pH dynamics, viable cell counts (CFU/ml), organoleptic evaluation, and comparative analysis with commercial yogurts. The optimized starter culture (B2) reduced the fermentation time to 5 hours and 40 minutes at 42 ℃, achieved an acidity of 110 °T, maintained pH within the range of 4.4–4.6, and ensured a viable cell concentration of 6×10^9 CFU/ml. The developed culture demonstrated superior microbiological and technological performance compared to commercial products. The results indicate the feasibility of industrial-scale production of indigenous starter cultures and highlight their strategic importance for the development of the dairy industry in Uzbekistan.

Keywords Lactic acid bacteria, Yogurt fermentation, Starter optimization, Thermophilic bacteria, Probiotic potential, CFU, Dairy biotechnology

1. Introduction

Microbiome profiling studies of naturally fermented dairy products in Central Asia have revealed a high diversity of indigenous lactic acid bacteria (LAB) communities and their unique adaptive characteristics, such as carbohydrate metabolism and acid tolerance [1]. Recent studies have also demonstrated that starter composition and fermentation conditions significantly influence metabolite production and the formation of health-promoting bioactive compounds. For instance, under co-fermentation conditions, the composition of starter cultures has been shown to significantly affect fermentation kinetics, acidity dynamics, and the synthesis of bioactive peptides [2]. In addition, probiotic LAB possess antibacterial, immunomodulatory, and antioxidant properties that may improve both product quality and consumer health [3,4].

The Government of Uzbekistan has set the development of the dairy industry as a strategic priority, aiming to increase milk processing capacity by 32% by 2026 [5]. Therefore, optimizing starter cultures based on local isolates would not only provide economic benefits but also ensure the production of health-promoting functional dairy products. According to laboratory data, yogurt products fermented with imported starter cultures usually contain 10^5–10^7 CFU/ml (colony-forming units per milliliter) of viable bacteria, whereas the functional probiotic threshold is $\geq10^6$ CFU/ml [6,7].

In recent years, yogurt has been recognized as one of the fastest-growing categories in the global dairy market. According to the International Dairy Federation [8], the global yogurt market expanded with an average annual growth rate of 5.2% between 2018 and 2023. At the same time, in Central Asia, including Uzbekistan, industrial yogurt production largely relies on imported starter cultures, which increases production costs and limits technological independence [1].

Yogurt is a fermented dairy product produced through the activity of thermophilic lactic acid bacteria, primarily Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. The metabolic cooperation between these microorganisms ensures rapid acidification, synthesis of flavor compounds such as acetaldehyde and diacetyl, and the formation of a gel structure, which determines the key physicochemical and sensory properties of yogurt [9,10,11].

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S. thermophilus grows rapidly during the initial stage of fermentation, reducing dissolved oxygen in milk and producing formate and CO_2, which stimulates the growth of L. delbrueckii subsp. bulgaricus. In turn, L. bulgaricus exhibits strong proteolytic activity, generating peptides and amino acids that provide essential nutrients for the growth of S. thermophilus [11,12]. Yogurt containing Lactobacillus acidophilus not only improves sensory and rheological properties but also enhances the probiotic and functional value of the product [13,2]. For this reason, it is widely used in the industrial production of functional and health-promoting yogurts.

During yogurt fermentation, the main biochemical processes include the following: hydrolysis of lactose into glucose and galactose by β-galactosidase; formation of pyruvate and lactic acid through glycolysis; destabilization of κ-casein micelles and gel formation due to decreasing pH; and inhibition of pathogenic and spoilage microorganisms as a result of acidification [14]. Strains capable of producing exopolysaccharides (EPS) improve yogurt texture, reduce syneresis, and enhance rheological stability [15]. EPS interact with the protein network and increase water-binding capacity. The optimal temperature range for thermophilic strains is 42–45 ℃, where enzymatic activity and glycolytic flux reach maximum levels [10]. However, titratable acidity above 120 °T may lead to excessive contraction of the protein network, resulting in a dense texture and overly sour taste.

Starter concentration directly affects fermentation kinetics. Increasing the inoculum level shortens the lag phase, increases the maximum growth rate (μmax), and leads to a higher final CFU/ml count [16,17]. For probiotic effectiveness, the product should contain at least 10^6–10^7 CFU/ml viable cells at the time of consumption [6,7]. Stabilizers such as STPP (sodium tripolyphosphate) interact with milk proteins through ion-exchange reactions, altering calcium balance and strengthening the casein network. This increases water-holding capacity, reduces syneresis, and improves product stability during storage [18]. In addition, stabilizer addition enhances protein–polysaccharide interactions and improves the rheological parameters of the gel.

Under the conditions of Uzbekistan, isolation and technological optimization of indigenous strains can reduce dependence on imported starter cultures and enable the development of highly efficient starters adapted to the characteristics of local raw milk. Therefore, the aim of this study was to develop a yogurt starter culture based on indigenous isolates and to evaluate its fermentation activity, microbiological stability, and potential for industrial application.

2. Materials and Methods

2.1. Microorganisms

The bacterial strains used in this study were Streptococcus thermophilus AWL, isolated from yogurt, and Lactobacillus delbrueckii subsp. bulgaricus AWL-D, isolated from strained fermented milk (suzma). These strains were selected due to their thermophilic characteristics and their technological suitability for yogurt fermentation.

2.2. Fermentation Conditions

Pasteurized cow's milk with a standardized fat content of 3.2% was used as the fermentation substrate. The milk was incubated at two different temperatures, 37 ℃ and 42 ℃, which were selected as the experimental fermentation conditions.

Several experimental variants were designed to evaluate the effect of starter composition and stabilizer addition on the fermentation process. The control sample consisted of milk without any additives or starter cultures. In the A1 variant, milk was inoculated with indigenous starter strains using a standard inoculum concentration. In the A2 variant, the starter culture was combined with STPP (sodium tripolyphosphate) as a stabilizer. In the B1 variant, the indigenous starter culture was applied at a higher cell concentration (double inoculum). Finally, the B2 variant consisted of the indigenous starter culture supplemented with STPP and a double cell concentration. These experimental conditions were used to determine the optimal combination for yogurt fermentation.

2.3. Analytical Methods

Titratable Acidity Determination

Fermentation time was determined by recording the incubation period after inoculation of milk with the starter culture. The endpoint of fermentation was defined as the onset of milk coagulation, indicated by the formation of a firm coagulum. Fermentation times were compared across different temperature conditions and starter culture variants, which served as control parameters for evaluating the fermentation process.

Titratable acidity (°T) was determined using the standard titration method. A defined amount of milk or yogurt sample was taken and titrated with 0.1 N NaOH solution using phenolphthalein as an indicator. The volume of NaOH consumed during titration was used to calculate the titratable acidity expressed in degrees Thorner (°T).

$$°T = (VNaOH \times NNaOH \times 9) / Vsample$$

In this context:

VNaOH – volume of NaOH consumed during titration (ml)

NNaOH – normality of the NaOH solution

Vsample – volume of the milk sample (ml)

pH measurement. The pH value was measured using a digital pH meter.

CFU/ml (Standard Plate Count Method).

The viable cell count was determined using the plate count method. Milk samples were serially diluted in a sterile diluent. The diluted samples were then inoculated onto semi-solid MRS culture medium and incubated at 37–42 ℃ for 24–48 hours. After incubation, the number of formed

colonies was counted.

The viable cell concentration was calculated using the following formula:

CFU/mL = (number of colonies × dilution factor) / plated volume (mL)

Organoleptic Properties.

The following attributes were evaluated by a sensory assessment panel: coagulum uniformity and gel structure, taste (sourness, sweetness), aroma, and syneresis (liquid separation). Each parameter was scored using a 5-point or 9-point scale, and the average values were calculated.

2.4. Statistical and Visual Analysis

ANOVA was used to determine differences among variants in terms of fermentation time, acidity, pH, CFU, and organoleptic scores. Statistical analysis was conducted based on triplicate experiments (mean ± SD).

3. Results and Their Analysis

During the implementation of the study, the following structure was developed and analyzed based on the established indicators (Table 1).

The study examined the effects of different starter cultures and incubation temperatures on milk fermentation. In the control samples, the coagulation time was 12 hours at 37 ℃ and 9 hours at 42 ℃. The application of starter cultures significantly reduced fermentation time, particularly in the B2 variant (double inoculum with STPP), where fermentation at 42 ℃ was completed in 5 hours and 40 minutes. This indicates that higher inoculum concentration and the presence of stabilizer substantially enhanced fermentation efficiency.

Table 1. Samples Selected for the Study

No	Sample	Composition	Preparation Method
1	Control	Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus	Culture suspensions were mixed with protective medium in a 1:1 ratio
2	Starter A1	Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus	Culture suspensions were mixed in a volume of 58 ml, then 20 ml of protective medium was added after centrifugation
3	Starter A2 (STPP)	Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus	Cultures were maintained in a nutrient medium containing STPP, mixed in a volume of 58 ml, then 20 ml of protective medium was added after centrifugation
4	Starter B1	Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus	Culture suspensions were mixed in a volume of 68 ml, then 30 ml of protective medium was added after centrifugation
5	Starter B2 (STPP)	Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus	Cultures were maintained in a nutrient medium containing STPP, mixed in a volume of 68 ml, then 30 ml of protective medium was added after centrifugation

Table 2. Fermentation Efficiency and Quality Indicators

No	Sample	Coagulation Time	Taste	Coagulum and Consistency	Titratable Acidity (°T)	pH	Colony Count (CFU/mL)
1	Control	37 ℃: 12 h / 42 ℃: 9 h	Rich taste, kefir like flavor	Liquid coagulum	70 / —	4.0 / 4.5	5×10^{6} / —
2	Starter A1	37 ℃: 9 h / 42 ℃: 7 h	Rich taste, kefir like flavor	Creamy coagulum	100 / —	4.5 / 4.4	8×10^{7} / —
3	Starter A2 (STPP)	37 ℃: 9 h / 42 ℃: 6 h	Rich taste, kefir like flavor	Creamy coagulum	110 / —	4.3 / 4.4	3×10^{8} / —
4	Starter B1	37 ℃: 9 h / 42 ℃: 6 h 20 min	Rich and pleasant taste	Creamy coagulum	105 / —	4.6 / 4.5	6×10^{7} / —
5	Starter B2 (STPP)	37 ℃: 8 h / 42 ℃: 5 h 40 min	Rich and pleasant taste	Creamy coagulum	110 / —	4.6 / 4.4	6×10^{9} / —
Notes: Coagulation times are shown according to the incubation temperature. The "—" symbol indicates that data are not available. The Taste and Coagulum/Consistency columns reflect the results of sensory evaluation.							

From a sensory perspective, all products retained kefir-like characteristics in taste, but those prepared with B1 and B2 starters exhibited a pleasant flavor and a firm, creamy gel structure, reflecting superior product quality and consumer acceptability. The analysis confirmed that the B2 starter (high cell concentration + STPP) demonstrated the most effective performance, reducing fermentation time at 42 ℃ in line with previous literature. Research has shown that mixed starter cultures generally promote faster acidification and acid production compared to single strains. Streptococcus thermophilus and Lactobacillus spp. act synergistically, increasing metabolic rates and shortening the time required to reach coagulation and target pH. Furthermore, fermentation temperature directly influences bacterial activity: thermophilic conditions around 40–42 ℃ accelerate the growth and acid production of S. thermophilus and L. bulgaricus, thereby reducing fermentation time [19].

Regarding titratable acidity (°T), the control sample reached 70 °T, A1 and B1 variants reached 100–105 °T, and A2 and B2 reached 110 °T, indicating higher acid production during fermentation. The pH values ranged between 4.0 and 4.6, with improved pH stability observed in samples containing STPP and higher inoculum concentrations. This high level of acidification corresponds to the metabolic activity of lactic acid bacteria, which convert lactose into lactic acid, thereby lowering pH. Studies have shown that pH decreases over time during fermentation and eventually stabilizes as elevated acidity slows bacterial metabolism. High titratable acidity also contributes to firm gel formation and creamy texture, consistent with the observed sensory characteristics [20].

Microbiologically, viable cell counts (CFU/mL) were 5×10^6 in control samples, while A2 and B2 variants reached 3×10^8 and 6×10^9 CFU/mL, respectively. These higher counts indicate increased bacterial proliferation during fermentation and enhanced probiotic potential. This result aligns with previous studies showing that mixed starter cultures maintain balanced Lactobacillus and Streptococcus populations, achieving high levels of viable cells [21]. High cell concentrations not only reflect fermentation efficiency but also improve the probiotic properties of the final product.

Overall, the results confirm that the B2 starter, combining a double inoculum with STPP at 42 ℃, provided the most optimal performance. Under these conditions, fermentation time was shortened, titratable acidity and pH remained stable, and both microbiological quality and organoleptic properties were enhanced. Products prepared with B1 and B2 starters exhibited a creamy texture, firm gel structure, and pleasant taste, consistent with literature reports indicating that mixed starter cultures increase acidification and proteolytic activity, thereby promoting flavor compound formation. Under the influence of lactic acid, casein aggregates and peptides interact to form a stable gel, while optimal acidification maximizes flavor and aroma development [21,20].

During the fermentation process, the pH of the milk decreased significantly over time, reflecting the activity of the starter bacteria in converting lactose into lactic acid. The initial pH was approximately 6.5, and during the early hours of incubation (lag phase), pH changes occurred slowly. Subsequently, during the exponential phase, the activity of the starter bacteria increased, and the pH dropped rapidly. Under optimal fermentation conditions (42 ℃ with double inoculum concentration), the pH stabilized in the range of 4.4–4.6, indicating the completion of fermentation and that the milk's acidity met quality requirements.

For comparison, variants with lower inoculum concentration and without STPP exhibited a slower pH decrease, resulting in a longer fermentation time. Moreover, the gradual and controlled decrease in pH contributed to the improvement of the gel structure and the organoleptic properties of the product. These results demonstrate that the composition of the starter and the fermentation temperature directly influence the efficiency of the fermentation process and that probiotic bacterial activity is enhanced under optimal conditions. This indicates that the locally produced starter used in this study is both effective and suitable for potential industrial application.

Based on the comparison with commercial products, microbiological analyses conducted after 10 days of storage indicated that the locally produced B2 starter culture exhibited considerably higher microbiological stability compared to several popular imported yogurt products. Specifically, the B2 starter retained 6×10^7 CFU/mL of viable microorganisms, whereas Musaffo Classic retained 4×10^6, Danone Nature 3×10^7, and Prostokvashino only 6×10^5 CFU/mL (Table 3).

Table 3. Microbiological Indicators and Storage Duration of Commercial Yogurts

Product Name	Country	Storage Duration	Cell Count (CFU/mL)
Musaffo Classic	Uzbekistan	10 days	4×10^6
Danone Nature	France	21–30 days	3×10^7
Prostokvashino Classic	Russia	20–28 days	6×10^5

These data indicate that there are significant differences in microbiological activity among commercially available yogurt products. The locally produced Musaffo Classic exhibited moderate levels of viable cells, demonstrating its competitiveness compared to imported products. At the same time, Danone Nature stood out for maintaining high levels of viable microorganisms (3×10^7 CFU/mL) over a 21–30 day storage period, reflecting its long-term shelf stability and high probiotic efficacy.

In contrast, the Russian-produced Prostokvashino Classic displayed relatively low viable cell counts (6×10^5 CFU/mL), indicating limited microbiological stability and reduced probiotic potential.

The locally produced yogurt based on indigenous starter cultures (Musaffo Classic) appears well-adapted to regional conditions and is capable of maintaining product quality during manufacturing. This provides opportunities to reduce dependence on imports, promote national production, and enhance economic efficiency.

The results suggest that additional scientific studies are necessary to further improve the microbiological quality of locally produced yogurt. In particular, research aimed at preserving and enhancing the activity of probiotic microflora should be prioritized. Moreover, the implementation of new technologies to improve product quality during extended storage and transportation is essential.

The high viable cell counts and long-term stability observed for the B2 starter confirm that local strains are well-suited for industrial applications. This high microbiological stability and proliferation efficiency offer significant potential to reduce import reliance, lower production costs, and strengthen the domestic market.

Furthermore, elevated CFU levels contribute to maintaining the organoleptic qualities of the product, including taste, texture, and consistency, over extended storage periods, thereby enhancing consumer acceptance. Consequently, locally developed starters are evaluated not only as technologically efficient but also as economically and industrially competitive products.

Future research is recommended to explore the genetic and biochemical characteristics of local strains, extend shelf life, and enhance probiotic activity. Advancements in these areas are expected to contribute substantially to the development of the dairy industry and the sustainable growth of the national economy.

4. Discussion

Incubation temperature and inoculum concentration are key factors influencing both the efficiency of the fermentation process and the quality of the final product. The results of this study demonstrated that the B2 starter, applied at 42 ℃ with a double inoculum concentration, represented the most optimal conditions, significantly reducing fermentation time and improving quality parameters. This setting maximizes the activity of thermophilic lactic acid bacteria and provides the potential for effective industrial application.

Moreover, the symbiotic relationship between S. thermophilus and L. bulgaricus strains constitutes one of the main mechanisms driving the fermentation process. Their cooperation accelerates lactose breakdown and elevates the acidity of the product, thereby enhancing the taste, texture, and microbiological quality of yogurt.

The addition of sodium tripolyphosphate (STPP) during fermentation further contributed to the strengthening of the protein network, stabilizing the gel structure and significantly reducing syneresis. This, in turn, improved the organoleptic and rheological properties of the final product.

The effect of double inoculum concentration on probiotic efficacy was also evident in the study. This parameter led to a significant increase in CFU counts, which plays a critical role in enhancing the functional and health-promoting properties of the product. A high concentration of viable probiotic microorganisms increases the likelihood of exerting beneficial effects during consumption and improves the overall health profile of the yogurt.

The widespread implementation of local starter strains in dairy production offers several positive economic and industrial benefits. Primarily, the use of local starters reduces dependence on imports, thereby substantially lowering the costs associated with purchasing commercial starter cultures. This, in turn, decreases production costs and enhances the overall competitiveness of the dairy industry.

Additionally, the application of local starters strengthens the technological independence of the dairy sector, as strains adapted to regional conditions help stabilize production processes. This fosters sustainable growth and promotes the development of innovative capacities within the dairy processing industry.

Furthermore, the advancement of local biotechnological potential creates opportunities for new scientific research, technological development, and product innovation, contributing to the long-term sustainable growth of the national economy.

Increasing the production of fermented dairy products using local starters, particularly functional yogurts and probiotic products, aligns with global trends in healthy nutrition. This not only meets domestic market demand but also expands export potential.

In conclusion, the industrial application of local starters enhances economic efficiency and plays a critical role in ensuring the ecological and social sustainability of the dairy sector. Therefore, continued research in this area and broader implementation in production processes should be considered a priority within state policy and industrial strategy frameworks.

5. Conclusions

The results of the study demonstrated that the B2 starter (42 ℃, double inoculum) reduced fermentation time by 45%, achieved a final titratable acidity of 110 °T, and maintained high viability with 6×10^9 CFU/mL of probiotic cells. The product exhibited excellent sensory quality, indicating that starters based on local strains have promising potential for industrial-scale application. These findings are of significant importance for the development of the national dairy industry and for reducing dependence on imported starter cultures.

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