MOLECULAR IDENTIFICATION OF NEW LOCAL STRAINS OF BIFIDOBACTERIA AND LACTOBACILLI USING 16S RRNA

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Abstract. *In light of the growing interest in using probiotic microorganisms in medicine and the food industry, there is an increasing need to develop effective methods for their identification and characterization. This study presents the results of molecular genetic analysis of newly isolated local strains of bacteria from the genera Bifidobacterium and Lactobacillus, obtained from samples collected in Uzbekistan. For identification purposes, methods of amplification and sequencing of the 16S rRNA gene were used, along with species-specific primers when necessary. The obtained nucleotide sequences were compared with NCBI databases, allowing for precise determination of the taxonomic position of the studied isolates. The research revealed the presence of both widely distributed and unique strains with potential for further application in the development of probiotic preparations. The acquired data contribute to expanding knowledge about the biodiversity of probiotic microflora and highlight the significance of local microbiological resources.*

Key words: *Identification of strains, local strains, bifidobacteria, lactobacteria, 16S rRNA, molecular genetic identification, probiotics, gene amplification.*

Introduction. In recent decades, there has been growing interest in studying the human microbiota as a key factor influencing health and organism development. Special attention is given to the intestinal microbiota of newborns, as it is during this period that the primary colonization of the intestines by microorganisms occurs, forming the metabolic and immune foundation for the entire lifespan. Among the most important representatives of the early microflora are bacteria of the genera Bifidobacterium and Lactobacillus, which possess pronounced probiotic potential. Strains of *Bifidobacterium* and *Lactobacillus* promote the fermentation of oligosaccharides, the synthesis of organic acids and B vitamins, and exhibit antagonistic activity against pathogenic and opportunistic microorganisms. Their metabolites modulate the immune response, reduce inflammation, and stimulate the maturation of the immune system in infants. Given these properties, they are widely used in the production of probiotic preparations, particularly for young children [6;8;9;10].

Nevertheless, the effectiveness of probiotics may depend on strain-specific properties, as well as the geographical and ethnic background that shapes the microbiota. In this context, the isolation and identification of local probiotic bacterial strains adapted to the biological characteristics of a specific region's population is becoming increasingly relevant. Studying the microbiota of newborns is particularly important, as it is at this age that the primary interaction between the immune system and microbes occurs.

In recent years, molecular genetic methods based on the amplification and sequencing of the 16S rRNA gene have gained widespread use in microbiological research, including the identification of probiotic bacteria of the Bifidobacterium and Lactobacillus genera. This approach enables a high degree of accuracy and reproducibility in determining the taxonomic position of bacterial isolates [1;3;5]. Furthermore, the use of 16S rRNA analysis facilitates comparison of the obtained data with extensive databases such as EzBioCloud and NCBI, which significantly simplifies the interpretation of results. This highly specific method allows for determining the taxonomic affiliation of microorganisms even in cases where cultivation is difficult or morphological characteristics are similar between different species.

In the present study, molecular genetic identification of two local strains isolated from the feces of healthy newborns in the Tashkent region was conducted to assess their belonging to probiotically significant species of Bifidobacterium and Lactobacillus.

Purpose of the study. To conduct molecular genetic identification of newly isolated local *Bifidobacterium* and *Lactobacillus* strains obtained from the feces of healthy newborns using 16S rRNA gene sequence analysis.

Materials and research methods. For the amplification of the 16S rRNA gene, validated universal primers were used:

[Forward Primer (Bifidobacterium) /5'-TGAAGGGTGGGGATGACGT-3',
Reverse Primer (Bifidobacterium): /5'-ACGGGCGGTGTGTACAAAG-3',
Forward Primer (Lactobacillus) /5'-GGGTGGTAATGCCGGATG-3',
Reverse Primer (Lactobacillus): /5'-CCACCGTTACACCGGGAA-3'].

These primers are widely used in studies for the identification of *Bifidobacterium* and *Lactobacillus* species. The choice of primers was based on publications by Jin et al. [4] and Matsuki et al. [7], which demonstrated high specificity and sensitivity of the respective primer pairs in detecting and differentiating target bacterial taxa. The study involved two strains: one representative of the genus Bifidobacterium and the other of the genus Lactobacillus. The strains were isolated from the feces of healthy 5-day-old newborns hospitalized in the maternity ward of the Zangiata District Medical Association in the Tashkent region. Molecular genetic identification was carried out using 16S rRNA gene sequencing. The nucleotide sequences were amplified using universal primers, followed by sequencing and comparison with reference databases (NCBI BLAST) to determine their taxonomic affiliation.

Results and discussion. The obtained results demonstrate the effectiveness of 16S rRNA profiling for the identification of newly isolated *Bifidobacterium* and *Lactobacillus* strains. However, it should be noted that factors such as sample processing method, DNA extraction technique, and primer selection for PCR can influence the accuracy of the results. These observations are consistent with the findings of Fouhy et al. [2], who showed that even minor variations in the protocol can significantly alter the composition of the detected microbiota. This highlights the importance of standardizing methodological approaches in microbiological and metagenomic studies.

During the molecular genetic identification of isolates obtained from local samples, the following data were obtained (Table 1). All isolated strains showed a high degree of similarity to specific reference strains, confirming their identity and affiliation with particular bacterial species.

1. *Bifidobacterium bifidum* CNCM I-4319 – The isolate identified as *Bifidobacterium bifidum* showed 100% identity with the reference strain, confirming the accuracy of molecular identification for this species. B. bifidum is well known for its important role in maintaining gut microbiota health and fermenting complex carbohydrates.
2. *Bifidobacterium animalis subsp.lactis* DSM 10140 – This strain showed 99% identity with the reference strain *B.animalis subsp.lactis*. This subspecies is commonly used in the production of probiotic supplements due to its ability to improve gut microbiota and support the immune system.
3. *Limosilactobacillus fermentum* EFEL6800 – Identified as *Limosilactobacillus fermentum*, with 98% identity to the reference strain. *L.fermentum* is known for its probiotic properties, including the ability to suppress pathogenic microorganisms and help maintain gut flora balance.
4. *Lactobacillus helveticus* DS3_8 – The isolate identified as *Lactobacillus helveticus* showed 100% identity with the reference strain. This species is used in cheese and other dairy product manufacturing and exhibits antimicrobial activity as well as the ability to support gastrointestinal health.
5. *Lacticaseibacillus rhamnosus* 1.0320 – Identified as *Lacticaseibacillus rhamnosus*, with 99% identity to the reference strain. This species is a well-known probiotic used for the prevention and treatment of diarrheal diseases and for enhancing the immune system.

The results of molecular identification confirm the high accuracy of the methods used for determining bacterial species and subspecies. All isolated strains demonstrated a high level of identity with reference strains, supporting their classification within the stated species. Notably, species such as Bifidobacterium bifidum and Lactobacillus helveticus, which showed 100% identity, exhibit strong potential for use in probiotic formulations, as evidenced by their well-documented beneficial properties. For instance, B. bifidum plays a crucial role in maintaining intestinal balance and may be used for the prevention of dysbiosis and other gastrointestinal disorders.

The isolate Bifidobacterium animalis subsp. lactis also showed a high level of identity and is a well-studied probiotic frequently used in the production of functional food products. This strain holds significant potential for improving gut microbiota and enhancing immune protection. The identification results of Limosilactobacillus fermentum and Lacticaseibacillus rhamnosus highlight the considerable diversity of local strains, which may possess unique probiotic properties. L. fermentum, with its antibacterial activity, and L. rhamnosus, with its proven immunomodulatory effects, could be utilized in the prevention and treatment of disorders associated with gut microbiota imbalance.

Thus, all isolated strains represent substantial interest for further research and the development of probiotic products. The obtained data may serve as a foundation for the creation of novel biotherapeutic strategies and functional foods based on local bacterial strains.

Table 1

BLAST analysis results of isolated strains of Bifidobacteria and Lactobacilli

Isolate	Closest Reference Strain	Species Identified	Sequence Identity (%)
1	*Bifidobacterium bifidum* CNCM I-4319	*Bifidobacterium bifidum*	100%
2	*Bifidobacterium animalis* DSM 10140	*B. animalis* subsp. *lactis*	99%
3	*Limosilactobacillus fermentum* EFEL6800	*Limosilactobacillus fermentum*	98%
4	*Lactobacillus helveticus* DS3_8	*Lactobacillus helveticus*	100%
5	*Lacticaseibacillus rhamnosus* 1.0320	*Lacticaseibacillus rhamnosus*	99%

The isolation of such strains from the intestines of healthy infants indicates their potential safety and physiological compatibility, which is especially important when developing probiotics for newborns.

It is important to note that the isolated strains were obtained from the feces of newborns in a maternity ward who had not received antibiotic therapy and were in satisfactory clinical condition. This points to natural colonization of the gut by potentially beneficial microorganisms and emphasizes the physiological relevance of the identified bacteria. From a practical perspective, local strains isolated from members of the regional population may be more effective when used in microbiota-targeted interventions, as they are adapted to the specific dietary habits, environmental conditions, and immune status of the population. Moreover, the confirmed taxonomic identity with industrial reference strains broadens the potential for their further use as probiotic agents.

Conclusions:

1. Molecular genetic identification of two newly isolated local strains obtained from the feces of healthy newborns was performed using 16S rRNA gene analysis.

2. All isolates were identified as *Bifidobacterium animalis subsp.lactis* with a 99% identity level, *Bifidobacterium bifidum* with 100% identity level, *Lactobacillus helveticus* with 100% identity level, *Lacticaseibacillus rhamnosus* with 99% identity level, and the last as *Limosilactobacillus fermentum* with a 98% identity level.

3. The obtained results indicate the presence of naturally occurring probiotic strains in the intestines of newborns from the Tashkent region, showing high similarity to industrially applied cultures.

4. The identified strains may be considered promising candidates for the development of regionally adapted probiotic formulations.

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