

INFLUENCE OF CONSUMPTION OF PROBIOTIC DAIRY YOGURT ON GUT MICROBIOME STRUCTURE

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ABSTRACT

This study shows the potential of probiotic dairy product to modulate the gut microbiota. The effect of the product on the intestinal microflora was determined by examining the fecal microflora of rats before and after 4 weeks of adding a fermented milk probiotic product to the diet. Structural changes in the faecal microflora were studied on the basis of sequencing V1-V3 hypervariable target region 16S rRNA gene. Sequencing results showed a decrease in microbiota biodiversity after taking a probiotic product. Nevertheless, enrichment of the microflora with butyrate-producing microorganisms *Lachnospiraceae*, *Ruminococcaceae*, *Lactobacillales* and depletion of *Porphyromonadaceae*, *Eggerthella*, *Romboutsia*, *Fusicatenibacter* and *Bacillus*, which are not belong to the order *Lactobacillales*.

Key words: Gut microbiome, rats, probiotic yogurt, microbial diversity, short-chain fatty acid

INTRODUCTION

A balanced human microbial ecosystem is an important factor in maintaining optimal health. These vital complex symbiotic relationships are beneficial for human's organism. Thus the intestinal microflora participates in the formation of human immunity from the moment of birth. Bacterial representatives of the microflora produce vitamins, antimicrobial substances, and organic acids with long and short chains in large quantities. The symbiotic fungal flora produces antibiotic substances, thereby preventing a possible infectious process. Whereas dysbiotic shifts can lead to a variety of consequences ranging from diarrhea to metabolic neurodegenerative diseases. Studies of the role of the microbiome and its structural features have led to an understanding of the effect of food products on the composition of the intestinal microflora. In this perspective, the consumption of fermented foods may be associated with the modulation of the functional capabilities of the gut microbiota. The most commonly consumed fermented foods are dairy product made on the basis of cow's milk. But the microorganisms used in the manufacture of these products may differ in their functional characteristics. For example, *Streptococcus thermophiles* serves as a starter culture for most of them. The use of probiotic microorganisms in the composition leads to an increase in the functional properties of the products. The consumption of such products, in turn, leads to a change in the composition and functionality of the intestinal microflora and the acquisition of additional ecosystem functions to maintain the ecological homeostasis of the host [1]. It is known that different probiotic products have different effects on the microflora with the formation of certain structural features. For example, consumption of Yakult (*Lactobacillus casei* strain Shirota) led to depletion of *Prevotellaceae* and enrichment of *Butyrivimonas* [2]. Feeding mice with *Lactobacillus rhamnosus* GG for 14 days resulted in enrichment of *Blautia* and *Lachnospiraceae* NK4A136_group [3]. Emiley A. Eloë-Fadrosh et al (2015), showed that consumption of a probiotic based on *Lactobacillus rhamnosus* GG for 28 days leads to the expression of ad-

hesion genes and bacterial motility in commensal intestinal bacteria *Roseburia* and *Eubacterium* motile gut species of butyrate producers [4].

The present research was aimed at studying the effect of fermented milk probiotic product enriched with bifidobacteria on microbial diversity and composition of rat intestines.

MATERIALS AND METHODS

The study conducted on mongrel laboratory rats. The experiments in total involved seven animals of both sexes, 3 months of age, with an average initial body weight of 212 ± 34.4 g. The rats were on the normal chow diet 7 days before the experiment and during the experiment. Drinking water was sterilized before use. To study the effect of own developed new probiotic bio-yogurt on the intestinal microflora of rats, the bacterial structure studied before and after taking the fermented milk product. The consumed product was manufactured on the basis of the starter consortium *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus paracasei* ssp. *paracasei*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium adolescentis* without the addition of sugar, preservatives, stabilizers and fillers according to standard technologies. The primary collection – the first control point (IA1) of fecal samples was carried out after 7 days of the normal chow diet. After that, a fermented milk probiotic active product introduced into the diet of the animals for 4 weeks. The second control point for collecting fecal samples (IA2) was conducted after 28 days of introduction of a probiotic product into the diet.

The study was approved by the local ethics committee of the Center for Life Sciences, National Laboratory Astana, Nazarbayev University, (approval No. 01-2021 dated 18/01/2021) (Nur-Sultan, Kazakhstan).

Sample processing

Fecal samples will be collect in a DNA/RNA Shield-Fecal Collection Tube (Zymo Research, R1101). Genomic DNA

from fecal samples will extract using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). Qualitative control of DNA isolation will perform by electrophoresis in 1% agarose gel. The concentration and purity of each DNA sample will be determine using an Invitrogen Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, California, United States). Sterile water served as a negative control.

Library preparation

Preparation of DNA libraries will be performed in accordance with the 16S Metagenomic Sequencing Library Preparation guide (part no. 15044223 rev. B, 2013) as follows: DNA amplification of the V1-V3 hypervariable target region of the 16S rRNA gene with the addition of the Illumina adapters, and contained the following sequences of the nucleotide pairs: 5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTACGGGNGGCAG-3' for the forward primer, and 5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' for the reverse primer. Purification of the reaction mixture will be carried out using Agencourt AMPure PCR purification kit (Beckman Coulter Inc. Beverly, Massachusetts, USA). Dual indices and Illumina sequencing adapters the Nextera XT Index Kit will be used. The library quality was quantified by Qubit dsDNA HS Assay Kit with the Qubit 2.0 fluorometer system (Invitrogen, Life Technologies, Grand Island, NY, USA). Library validation will be conducted using Agilent DNA 1000 Kit and Agilent Technologies 2100 Bioanalyzer. For cluster generation and sequencing, libraries will be pooled are denatured with NaOH, diluted with hybridization buffer, and then heat denatured before MiSeq sequencing.

Processing of sequencing data

The LotuS2 (Less OTU Scripts 2) used to process 16S amplicon sequencing data from raw reads into taxon density ta-

bles. Demultiplexing, quality filtering, and dereplication of reads are implement using a simple demultiplexer (sdm). Chimeras will remove using algorithms for detecting chimeric sequences UCHIME. Taxonomic post-processing of amplicon sequences in LCA with sequence clustering UPARSE performed using SILVA, 16S rRNA gene database.

Statistical analysis

Analysis of alpha diversity to assess the abundance of the community and the calculation of biodiversity Shannon, Simpson, Chao1 and Ace indexes, as well as the construction of taxonomic distribution at the phylum and genus level were performed using phyloseq package (v.1.24.2) [5]genetics, phylogenetics, multivariate statistics, visualization and testing. With the increased breadth of experimental designs now being pursued, project-specific statistical analyses are often needed, and these analyses are often difficult (or impossible). All graphs were generated using ggplot2 (v.3.0.0) [6].

Non-parametric Mann-Whitney (MW) and Kruskal-Wallis (KW) tests were used when comparing two or more Shannon index comparison groups respectively. The abundance of taxa was calculated using ANOSIM and PERMANOVA statistical tests upon UniFrac weighted and unweighted distances using the vegan package (v.2.5.3) [7]. The validity of beta-diversity statistics was tested using BETADISPER.

RESULTS

This research studied the effect of a fermented milk probiotic active product on the intestinal microflora of rats. Prior to the study, the animals fed solid food without the addition of dairy products to eliminate mixed factors that could potentially affect the results of the analysis. To determine the ability to modulate fecal microflora, a fermented milk probiotic product was introduced into the diet of animals for 4 weeks

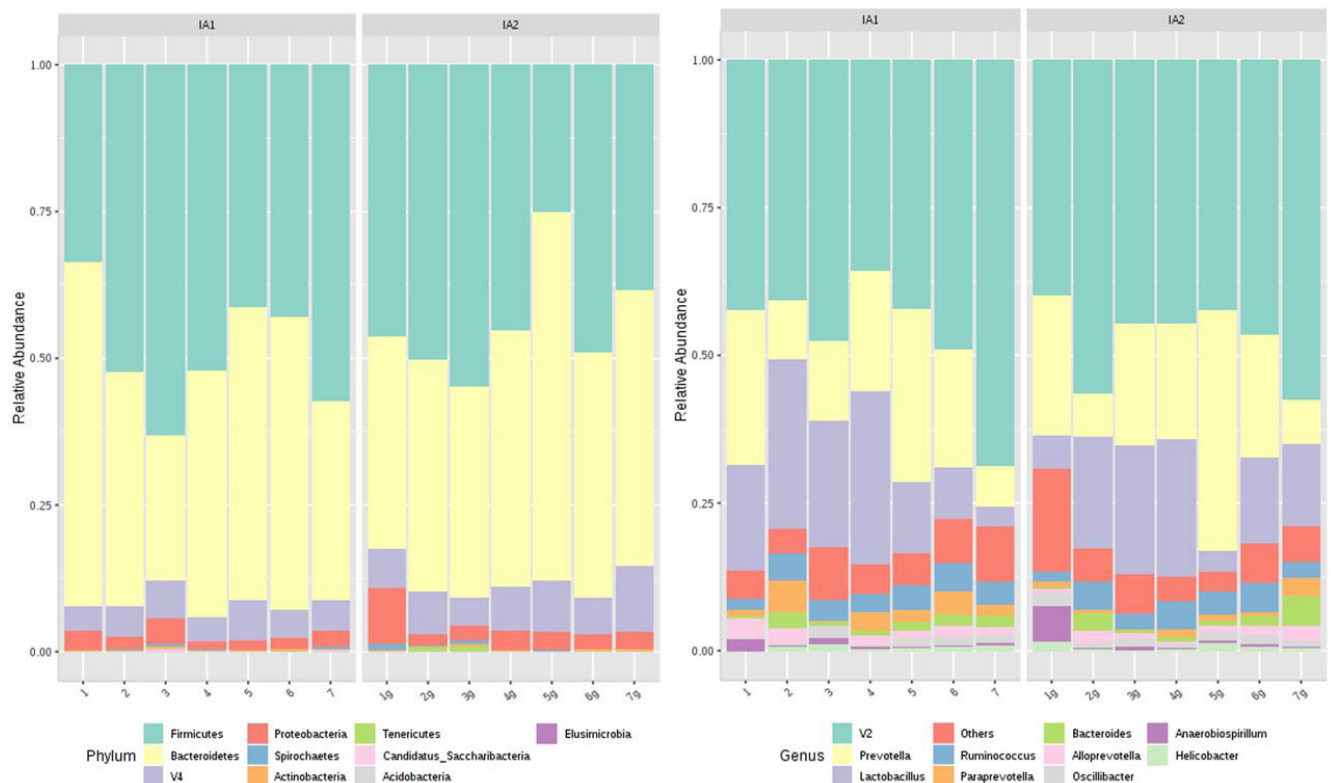


Figure 1. The abundant and distinct bacteria (phylum and genus level) in rat's fecal microbiota after consumption of a probiotic dairy product.

in an amount of 5 ml per animal per day. The samples were collected in sterile laboratory tubes, separately from each rat and immediately frozen at -20°C . The nucleotide sequence V1 – V3 region of the 16S rRNA gene used for microbiome analysis. The depth of coverage was at least 36,700 readings per sample. All sequences were compared with the SILVA database. The analysis of alpha diversity did not demonstrate fundamental differences between the studied groups (Figure 1) both at the taxonomic level of phylum and at the level of genus.

The composition of the intestinal microflora studied in fecal samples before and after consumption of the tested product. The relative abundance of bacterial taxa assessed at different taxonomic levels. The main taxa at the phylum level were *Firmicutes*, *Bacteroidetes*, *taxon V4* and *Proteobacteria*. If it drops below the level, the structure of the microbiome demonstrates the following predominant genus: *Prevotella*, *Lactobacillus*, *Ruminococcus* and other. As figure 1 shows, there are no fundamental differences before and after consumption of the product at these taxonomic levels. The only exception is the taxon *Lactobacillus*, the average abundance of which increased after taking the product ($p < 0.05$). Biodiversity indices showed a decrease in the relative diversity of bacterial taxa in fecal samples after ingestion of the product under study (Figure 2).

The Shannon and Simpson indices indicate lower community diversity, the results show that the fecal microbial diversity in the IA1 group was somewhat greater than in the IA2 group. This result was unexpected for us because it is believed that fermented milk products are beneficial for human health and increase the biodiversity and functional role of the intestine. The assessment of the biodiversity of the microbial flora of faeces based on the Shannon and Simpson biodiversity indices demonstrated differences. Figure 2a shows that the relative abundance of microbial flora after taking a probiotic product in fecal samples decreases. In addition, we observed that shift changes in the Shannon index between baseline bacterial community and bacterial community after ingestion of the fermented product were positively associated with differences (Figure 2b), indicating that a more marked change in community structure was associated with diversity within

the community more significantly. This result, in our opinion, related to: 1) the selective effect of the consumed product, which is a nutrient medium for a certain intestinal flora; 2) the antimicrobial pressure of the starter bacterial consortium. This is indirectly confirmed by the β -diversity analysis (Figure 2b), which shows the grouping of fecal samples after taking a probiotic dairy product. Nevertheless, the bacterial community is similar between both groups, which indicates that the microbiome core under the influence of the product introduction factor does not lead to a significant change in the composition of the microflora, but only enriches or depletes certain taxonomic groups.

To differentiate the changed taxa between the two groups, we used the approach of constructing heat trees. To do this, we first calculated the difference for each taxon between the sample communities. To visualize the differences, we used a divergent color scheme; purple shows a decrease in the relative abundance of a particular taxon, while light blue, in contrast, shows enrichment.

Figure 3 demonstrates an increase in the abundance of certain taxonomic groups under the influence of the consumed probiotic product. Thus, the increase was subjected to *Proteobacteria* (*Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*) *Prevotellaceae* (*Prevotella*, *Paraprevotella*), *Lachnospiraceae* (*Roseburia*), *Ruminococcaceae* (*Clostridium_IV*, *Oscillibacter*, *Flavonifractor*, *Pseudoflavonifractor*, *Papillibacter*, *Ruminococcus*), *Lactobacillales* (*Lactobacillus*, *Streptococcus*). Whereas, *Porphyromonadaceae*, *Eggerthella*, *Romboutsia*, *Fusicatenibacter* and *Bacillus* not belonging to the order *Lactobacillales* were depleted.

DISCUSSION

In this study, the effect of a probiotic fermented dairy product on the intestinal microflora was evaluated in laboratory mongrel rats. Using a healthy group of laboratory animals in our study allowed us to control both dietary and environmental factors, which are immensely difficult to control in human studies. The study did not show fundamental changes in the compositional structure of the intestinal microbiota of laboratory animals, but at the same time minor changes were still present. The present study shows that the consumption

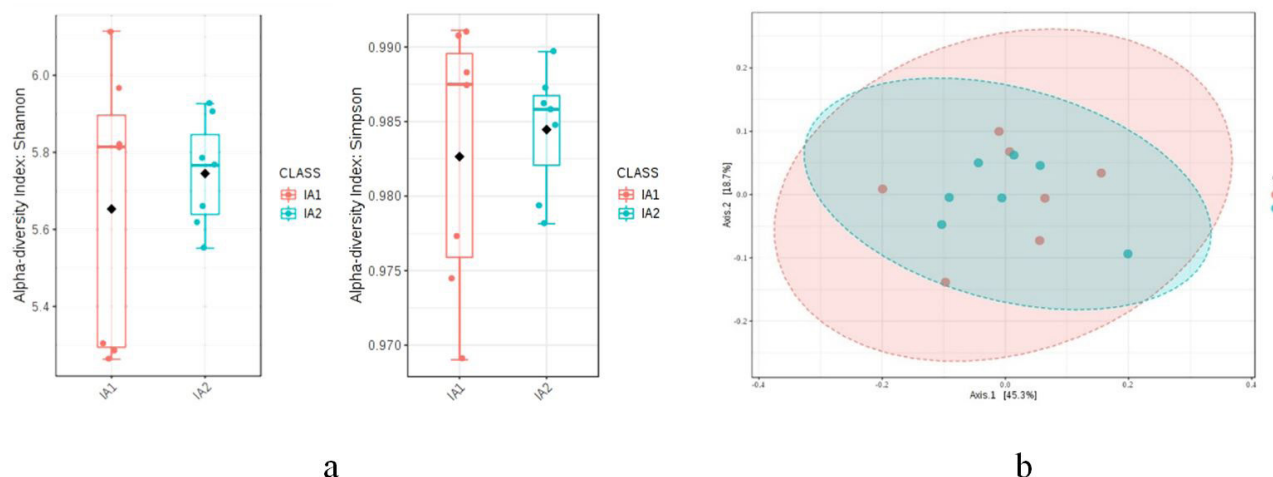


Figure 2. Structural changes in fecal microflora after consumption of a probiotic dairy product. a - evaluation of the alpha diversity of the intestinal microbiota before and after consumption of milk probiotic product. Boxplots display the median value, the first (25%) and third (75%) quartiles with whiskers from 1.5 IQR (interquartile range) minimum to maximum; b - Principal coordinate analysis (PCoA) plot in different axis PCoA1 (Axis 1) and PCoA2 (Axis 2) respectively explained 45.3 and 18.7% of the variance of the abundance of gut microbiota at the genus level.

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ВЛИЯНИЕ ПОТРЕБЛЕНИЯ ПРОБИОТИЧЕСКОГО МОЛОЧНОГО ЙОГУРТА НА СТРУКТУРУ МИКРОБИОМА КИШЕЧНИКА

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АБСТРАКТ

Это исследование показывает потенциал пробиотического молочного продукта, модулировать микробиоту кишечника. Влияние продукта на микрофлору кишечника определялось посредством исследования фекальной микрофлоры крыс до и после 4 недельного добавления в рацион ферментированного молочного пробиотического продукта. Структурные изменения в микрофлоре фекалий изучались на основе секвенирования V1-V3 гипервариабельного целевого региона 16S рРНК гена. Результаты секвенирования показали снижением биоразнообразия микробиоты после приема пробиотического продукта. Тем не менее выявлено обогащение микрофлоры бутират продуцирующими микроорганизмами и *Lachnospiraceae*, *Ruminococcaceae*, *Lactobacillales* и *истощение Porphyromonadaceae*, *Eggerthella*, *Romboutsia*, *Fusicatenibacter* и *Bacillus не относящиеся к порядку Lactobacillales*.

Ключевые слова: Микробиом кишечника, крысы, пробиотический йогурт, микробное разнообразие, короткоцепочечные жирные кислоты

ПРОБИОТИКАЛЫҚ СҮТ ЙОГУРТЫН ТҮТЫНУДЫҢ ІШЕК МИКРОБИОМЫНЫҢ ҚҰРЫЛЫМЫНА ӘСЕРІ

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ТҮЙІН

Бұл зерттеу пробиотикалық сүт өнімінің ішек микробиотасын модуляциялау мүмкіндігін көрсетеді. Өнімнің ішек микрофлорасына әсері ашытылған сүтті пробиотикалық өнімді 4 аптадан кейін егеуқұйрықтардың фекальды микрофлорасын зерттеу арқылы анықталды. Нәжістің микрофлорасындағы құрылымдық өзгерістер 16S рРНК геннің V1-V3 гипервариабельді мақсатты аймақ секвенирлеу негізінде зерттелді. Секвенирлеу нәтижелері пробиотикалық өнімді қабылдағаннан кейін микробиотаның биоалуантүрлілігінің төмендегенін көрсетті. Дегенмен, бутират микрофлорасының *Lachnospiraceae*, *Ruminococcaceae*, *Lactobacillales* өндіретін микроорганизмдермен байытылуы және *Porphyromonadaceae*, *Eggerthella*, *Romboutsia*, *Fusicatenibacter* және *Bacillus*-тің сарқылуы анықталған.

Кілтті сөздер: Ішек микробиомы, егеуқұйрықтар, пробиотикалық йогурт, микробтардың әртүрлілігі, қысқа тізбекті май қышқылдары