Food Bioscience 42 (2021) 101167

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Gut modulation of dysbiosis induced by dextran sulfate sodium

Samat Kozhakhmetov [a,c,*], Dmitriy Babenko [b,d], Saniya Kozhakhmetova [c,g], Altynay Tuyakova [a], Madiyar Nurgaziyev [a], Ayaulym Nurgozhina [a], Nurislam Muhanbetganov [a,c], Laura Chulenbayeva [a], Shynggys Sergazy [a,h], Alexander Gulyayev [a,h], Baimakhan Tanabayev [e], Timur Saliev [f], Almagul Kushugulova [a]

[a] Laboratory of Human Microbiome and Longevity, Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Z05H0P9, Nur-Sultan, Kazakhstan
[b] Research Center Karaganda Medical University, M01K6T3, Karagandy, Kazakhstan
[c] SaumalBioTech, Z05H0K9, Nur-Sultan, Kazakhstan
[d] Innovative Center ArtScience, Z11F5A9, Nur-Sultan, Kazakhstan
[e] South-Kazakhstan Medical Academy, X08F5X5, Shymkent, Kazakhstan
[f] S.D. Asfendiyarov Kazakh National Medical University, A05H2A6, Almaty, Kazakhstan
[g] National Center for Biotechnology, Z05K8D5ó, Nur-Sultan, Kazakhstan
[h] Nazarbayev University, Z05H0P9, Nur-Sultan, Kazakhstan

ARTICLE INFO

Keywords:
DSS-Induced colitis
Gut microbiome
Microbial diversity
Biological product
Rats

ABSTRACT

Inflammatory bowel disease is one of the serious burdens of clinical medicine and healthcare. This study investigated the potential of a biological product based on mare's milk and metabolites of symbiotic microflora for modulation of intestinal microflora affected by dextran sulfate sodium (DSS)-induced dysbiosis. Symbiotic microflora was isolated from the stool of healthy volunteers. Lysates for the production of short-chain fatty acids of screened microorganisms were mixed with mare's milk. The activity of the biological product was evaluated on the DSS model of induced colitis. Histological changes in the intestinal epithelium were determined. The structure of the microbiome was evaluated based on the analysis of 16S rRNA microbial sequences. Histological examination of rat intestinal tissues after application of the biological product showed reduced infiltration of granulocytes, macrophages, and lymphocytes. The results of sequencing demonstrated a decrease in the biological diversity of microbiota affected by colitis. The full recovery was observed after 21 days of the application of the biological product. The product induced the structural changes of the microbiome damaged by DSS. Likewise, the number of the pathogenic intestinal microflora was decreased Representatives of SCFA producing bacteria increased concentrations of genus *Lactobacillus*.

1. Introduction

Intestinal microbiome is a highly diversified heterogeneous ecosystem that performs and regulates the most important physiological and immunological functions. At the same time, there is a range of reports indicating the role of the intestinal microbiome in the pathogenesis of various diseases, such as obesity, diabetes mellitus, inflammatory bowel disease, non-alcoholic hepatitis, and cardiovascular diseases (Abboud & Papandreou, 2019; Yoshida et al., 2018).

Moreover, the intestinal microbiota plays a crucial role in the protecting the body from the negative effects of the external environment. During the life process, microbiota performs many important functions such as antimicrobial activity (to protect the organism from various infections), stimulation immune defense, the synthesis of secondary fatty acids, and the production of vitamins. One of the most important functions is the synthesis of short-chain fatty acids (SCFA). SCFAs is a major class of metabolites produced by bacteria in the large intestine by saccharolytic fermentation of carbohydrates or protein fermentation (Roy et al., 2006). The main types producing butyrates are *Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii and R. bromii*. *Bacteroidetes* are present in a smaller proportion and include the following main representatives of *Alistipes, Akkermansia, Bacteroides, Parabacteroides, Porphyromonas*, and *Prevotella* that produce mostly propionates and acetates (Macfarlane & Macfarlane, 2003). The potential role of SCFAs as signaling molecules that regulate glucose homeostasis in the liver has been also reported (Morrison & Preston, 2016).

* Corresponding author. Kabanbay batyr avenue 53, Z05P1T1, Kazakhstan.
E-mail address: sskozhakhmetov@gmail.com (S. Kozhakhmetov).

https://doi.org/10.1016/j.fbio.2021.101167
Received 31 December 2020; Received in revised form 16 March 2021; Accepted 15 April 2021
Available online 8 June 2021

Table 1
Short chain fatty acid producers.

Sample	Production of short chain fatty acids, mmol/l			
	acetate	butyrate	propionate	lactate
Bifidobacterium adolescentis kz1d1	<10	>10	>10	<10
Bifidobacterium adolescentis kz1d11	>10	>10	>10	<10
Eubacterium hallii kz2f1	–	<20	<10	–
Coprococcus eutactus kz2f6	–	<15	–	–
Faecalibacterium prausnitzii kz4d9	–	<20	–	–
Eubacterium rectale kz4e5	–	<20	<10	–
Enterococcus durans kz5b9	<20	<20	<10	>5
Enterococcus durans kz5b10	<20	<20	<10	>5
Alistipes shahii kz6A6	<10	<15	–	–

Table 2
The total histomorphometric score of the severity of colitis.

Animal groups	Histological severity score of colitis
HC (n = 4)	0,06 ± 0,05
Water (n = 4)	9,44 ± 0,81[a]
SP (n = 4)	4,44 ± 0,45#
5-ASA (n = 4)	4,10 ± 1,36#

Note: # - p <0,05 when compared to Water.
[a] - p <0,05 when compared to HC.

It was found out that a decrease in the diversity of the microflora of the large intestine correlates with the development of many disorders, for example, metabolic syndrome (Karlsson et al., 2013), type 2 diabetes (Lapthorne et al., 2013), inflammatory bowel disease and colorectal cancer (Ahn et al., 2013). Destruction of the microbial ecosystem structure is of great importance in the pathogenesis of various diseases, for example, for the development of inflammatory bowel syndrome, Crohn's disease, and ulcerative colitis (UC). In fact, the patients with ulcerative colitis suffer from the impaired diversity and stability of the intestinal microbiome associated with a decrease in Firmicutes and an increase in the number of *Bacteroidetes* and other facultative anaerobes (Shen et al., 2018). As a result of ulcerations, the intestinal wall ceases to be a normal niche for the living of microorganisms and performs a barrier function.

This study aimed at investigating the impact of metabolites of microbial cultures and mare's milk on the modulation of rat intestinal microbiome.

2. Materials and methods

Recruiting healthy volunteers was carried out in the period from December 2018 to April 2019. Samples collection was carried out according to the procedures described previously in the literature (Weinstein et al., 2018). 6 stool samples were collected. All volunteers were introduced to the study and completed informed consent for the study. The study was approved by the local Ethics committee of the Center for Life Sciences, PI "National Laboratory Astana", Nazarbayev University (extract from the protocol No. 20 dated 09/22/2017).

2.1. Bacteriological study

Stool specimens were delivered to the laboratory in the transport medium no later than 2 h after defecation. Serial dilutions of feces were carried out in sterile saline. Sowing was performed on the following culture media: Wilkins Chalgren Agar, BSM Agar, Meat Infusion Agar, Brain Heart Infusion Agar, Bifidobacterium Agar, MRS Agar, Reinforced

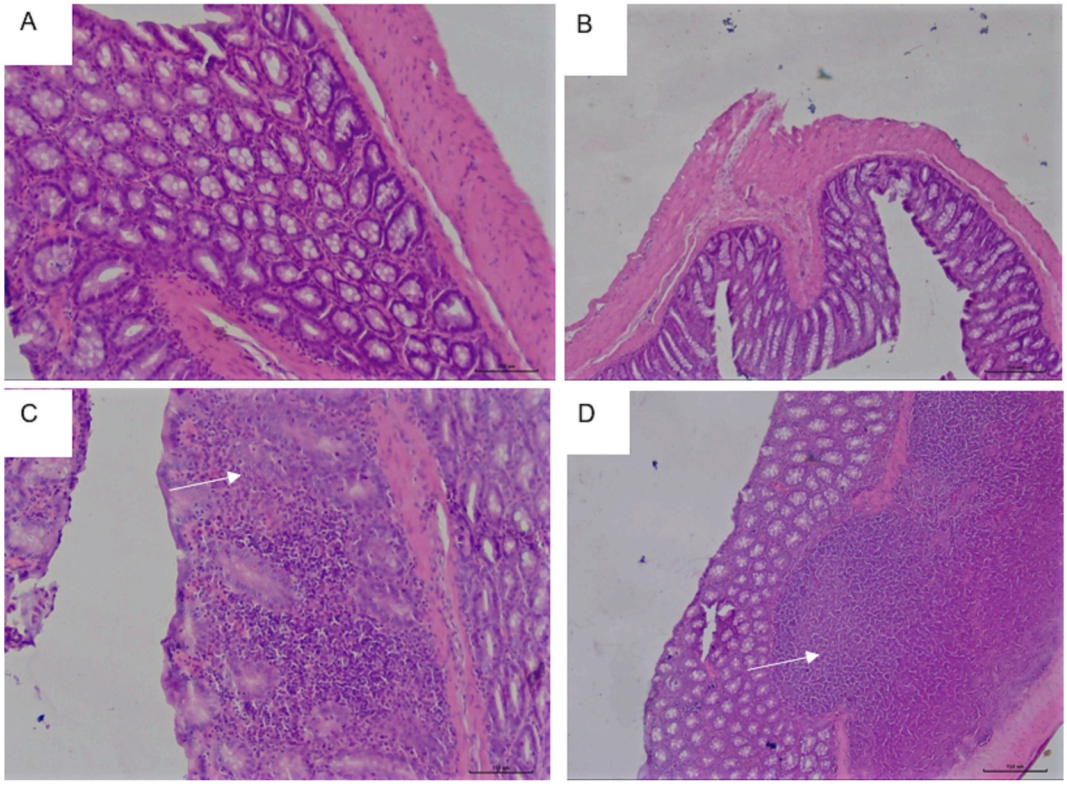

Fig. 1. Micrographs of representative sections of the colon stained with hematoxylin and eosin in a healthy control group (without colitis model) (A, B) and in the group of animals receiving active treatment with a biologically active substance based on mare's milk and microbial lysate (C, D). Magnification x100. A) healthy epithelium, crypts are presented in cross section. B) healthy epithelium, crypts are presented in oblique and longitudinal sections. C) diffuse infiltration of inflammatory cells in the mucosa, with epithelial hyperplasia and loss of goblet cells, deformation and destruction of crypts, marked by swelling of the mucosa. D) focal inflammatory granulocytic cell infiltrates in the submucosa.

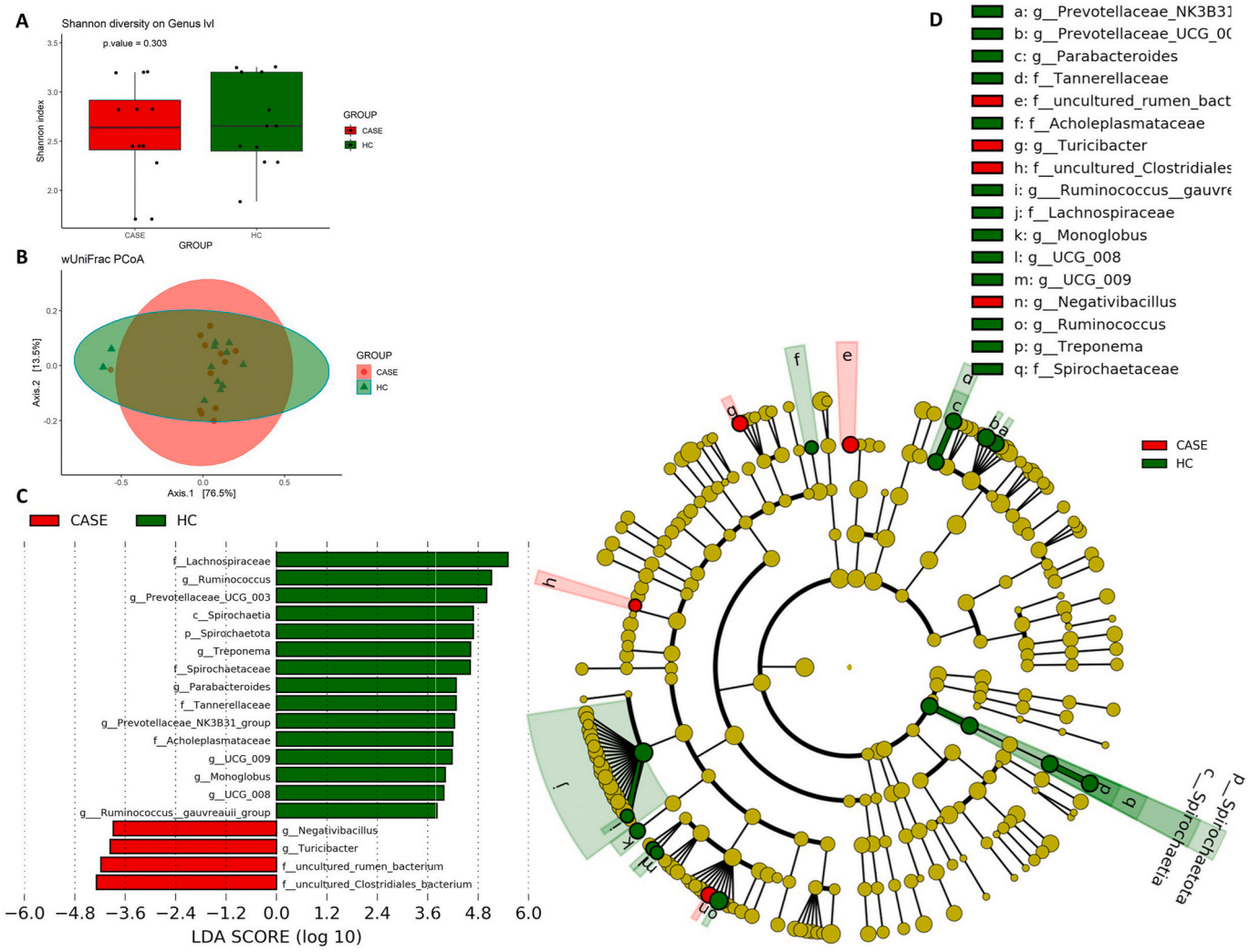

Fig. 2. Effect of DSS on the rat gut microbiome. A) Alpha diversity (Shannon index) was calculated at the genus level and displayed as box plot; B) Beta Diversity (HC – healthy control, CASE – DSS induced dysbiosis), Principal Coordinate Analysis Unweighted UniFrac Distance [PERMANOVA] p-value: 0.698; C) Distribution histogram based on LDA, with a log LDA score above 2. Significant taxa (p-value <0.05) were labeled and annotated with tags, genus level. D) Cladogram.

Clostridial Agar, LB agar. Incubated was carried out on Petri dishes under anaerobic conditions (CO_2 - 13.0%, N_2 - 86.5%, O_2 - 0.5%). Single bacterial colonies were seeded into pure culture media. The purity of the cultures was determined by microcopy Axio Imager 2 (ZEISS).

2.2. Identification

Single colonies were inoculated into appropriate culture media and were identified using Maldi Bioyper. Samples for MALDI-TOF MS analyses were prepared according to (Schulthess et al., 2014). Briefly, a fresh single colony was transferred to a polished steel target MSP 96, then 1 μl of a saturated solution of a-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonik) was added and left to dry at room temperature.

2.3. Determination of short chain fatty acids

Precipitation of cultures was carried out by centrifugation at 6000 rpm for 5 min. The supernatant was collected in clean tubes. As the form used the medium corresponding to the cultivation. Preparation of standards: desired concentration of standards (Propionic acid, 94425-1 ML-F, Sigma-Aldrich; Butyric acid 19215-5 ML, Sigma-Aldrich; Lactic acid PHR1215-3X1.5 ML Sigma-Aldrich; Acetic acid PHR1748-3X1,5 ML, Sigma -Aldrich). Stock concentrations of 200 mg/L standards in acetonitrile were prepared. 7.5 μl (1.5 μg), 15 μl (3 μg), 22.5 μl (4.5 μg), 30 μl (6.0 μg) of standards for lactic acid were prepared from the runoff. Derivatization was carried out by adding 20 μl of N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-Butyldimethylchlorosilane to the standards and test samples. Test tubes with standards and samples were kept for 3 h at 70°C. For butyric, propionic, and acetic acids: 2 μl (4 μg), 5 μl (10 μg), 10 μl (20 μg) and 15 μl (30 μg). After drying the products under nitrogen, 100 μl (9000 μg) of ethyl acetate was added to resuspend the contents, the tubes were shaken for 1 min before GC/MS analysis. For the analysis of SCFAs a device was used GC/MS Agilent 5977B GC/MSD quadrupole mass spectrometer.

2.4. Preparation of study products

The study product was obtained by mixing bacterial cultures followed by lysing. The supernatant of lysed cultures was obtained by centrifugation at 12,000 rpm for 60 min. The resulting substance was mixed with mare's milk and freeze-dried.

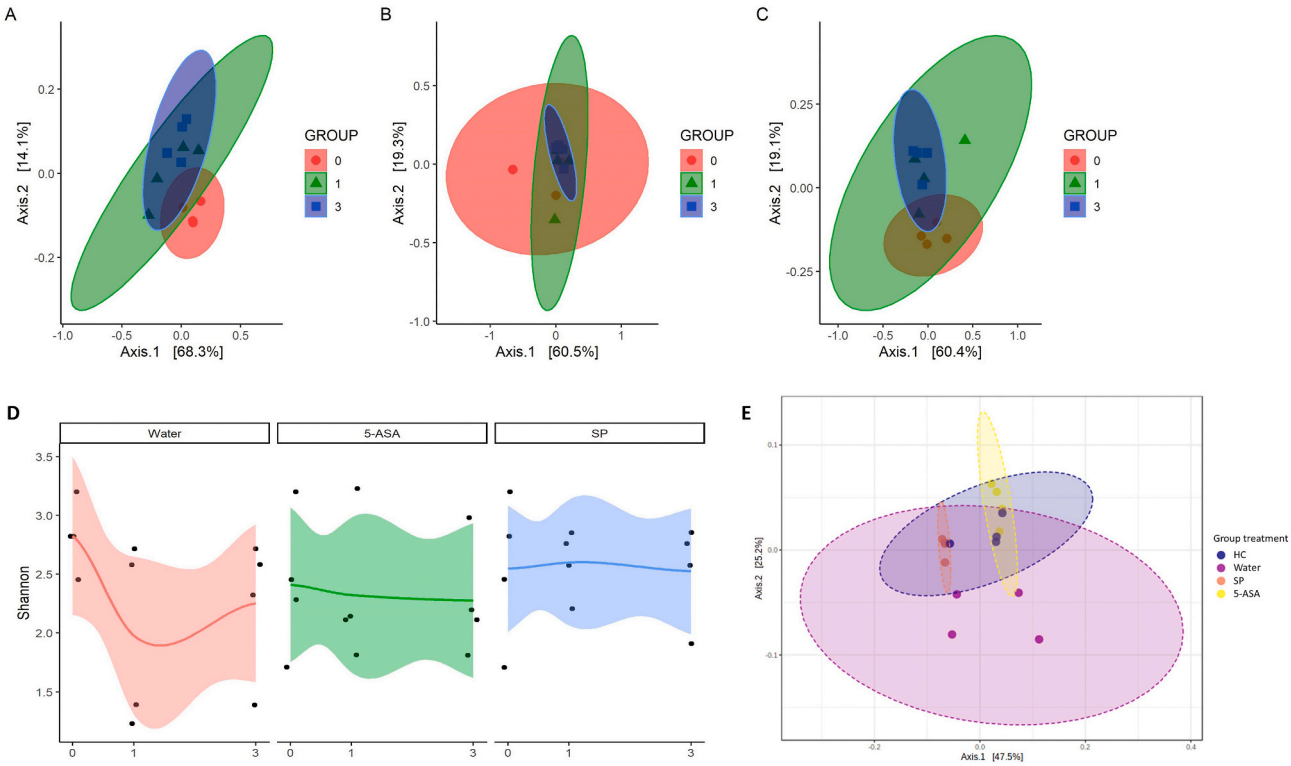

Fig. 3. The effect of the introduction of sodium dextran sulfate on the intestinal microflora of rats. A,B,C) Principal Coordinate Analysis Unweighted UniFrac Distance for Water (A), 5-ASA(B), SP(C) group in dynamic from initial to 3 week; D) Shannon index; E) Principal Coordinate Analysis Unweighted UniFrac Distance for all groups after 3 week treatment [PERMANOVA] F-value: 4.4685; R-squared: 0.52766; p-value < 0.001.

2.5. Animal study

The study was carried out on 16 laboratory animals (male Wistar rats with an average body weight range of 250–280) in the vivarium of National Center for Biotechnology (Nur-Sultan, Kazakhstan) with a standard ration and care. Rats were placed in separate cages in a room free of pathogens and for acclimatization 7 days before the start of the experiment. During acclimatization and experiment, rats consumed a standard commercially available chow. The animals were kept and the experiments conducted in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and the ethical principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe, 2006).

2.6. Experimental colitis model

Colitis in male rats was reproduced using a 10% DSS solution (Dextran sodium sulfate, MW - 40 kDa, Sigma Aldrich) (Chassaing et al., 2014), which was administered to the rats intra-gastrically using a probe for 7 days in a volume of 5 ml. The studied product to study the efficacy against microbial dysbiosis in the DSS-induced colitis model in rats was administered intra-gastrically at a dose of 500 mg/kg of animal body weight, once a day for 21 days after the end of a 7-day course of taking a 10% solution DSS. Healthy animals - HC (without colitis) received drinking water intra-gastrically instead of 10% DSS for 7 days and then another 21 days instead of treatment (n = 4). Experimental group animals – Water (with colitis) received a 10% dextran sulfate sodium (DSS) solution for 7 days (n = 4). Experimental group animals –SP (with colitis) received a 10% dextran sulfate sodium (DSS) solution for 7 days, and study product intra-gastrically at a dose of 500 mg/kg body weight once per day for 21 days (n = 4). Comparison group animals 5-ASA (with colitis) received a 10% DSS solution for 7 days and as a treatment the 5-ASA (5-aminosalicylic acid) intra-gastrically at a dose of 100 mg/kg of animal body weight once for 21 days (n = 4). Rats were removed from the experiment by an overdose of carbon dioxide (Hewett et al., 1993). Fecal samples were collected before and after the experiment and tested for consistency and color. Likewise other tested parameters were analyzed, including intestinal permeability and body weight.

2.7. Histology study

For histological examination, 6 samples of intestinal tissue were taken from each individual from 0.5 cm to 1.0 cm long, indicating the area from which the sample was taken (i.e. proximal, middle or distal) located at an equal distance from each other, which were fixed in 10% formalin at 4 °C for at least 24 h. An average histo-morphometric assessment of the severity of colitis was calculated for each sample. Twenty-four hours later, intestinal tissue samples were washed with water and dehydrated with a series of increasing concentrations of alcohol (70%, 90%, 95%, and 100%), then immersed in xylene and embedded in paraffin blocks. Tissue sections 5 μm thick were made on a Leica SM 2000R sled microtome. Slices were dewaxed in xylene, then hydrated in a series of decreasing alcohol concentrations (100%, 96% and 70%).

Histopathological and morphometric analyzes were performed on the transverse and longitudinal sections of the intestinal tissue of each animal, morphometric calculations were carried out on 10 fields of view.

A total histo-morphometric score for the severity of colitis was obtained by summing up the score on eight points (“inflammatory infiltrate”, “goblet cell loss”, “hyperplasia”, “crypt density”, “muscle thickness”, “submucosal infiltration” the presence or absence of crypt abscesses and ulcers) using a scoring system provided by Koelink et al. (2018). When calculating the “inflammatory infiltrate” score for assessing the relative cellularity in tissues, cell infiltration, including neutrophils, eosinophils, monocytes, plasma cells and lymphocytes in

Phylum	Genus	Treatment	Initial [IQR]	1 week [IQR]	3 week [IQR]	Coef	Pval
Actinobacteriota	Collinsella	WATER	0.00 [0.00;0.00]	0.03 [0.02;0.03]	0.02 [0.02;0.03]	↑ 0.646	**0.0355**
		5-ASA	0.01 [0.00;0.01]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	-0.632	0.1429
		SP	0.01 [0.00;0.01]	0.02 [0.01;0.05]	0.02 [0.01;0.05]	0.38	0.4121
	Rothia	WATER	0.02 [0.01;0.02]	0.01 [0.00;0.02]	0.00 [0.00;0.01]	-0.391	0.1812
		5-ASA	0.02 [0.00;0.08]	0.02 [0.00;0.03]	0.00 [0.00;0.00]	↓ -1.2	**0.0183**
		SP	0.01 [0.00;0.02]	0.01 [0.00;0.01]	0.01 [0.00;0.01]	-0.226	0.6277
Bacteroidota	Alloprevotella	WATER	0.38 [0.36;0.62]	1.18 [0.27;2.12]	1.24 [0.37;2.12]	0.161	0.6560
		5-ASA	0.29 [0.20;0.57]	0.25 [0.13;0.67]	0.44 [0.35;0.80]	0.219	0.4679
		SP	0.35 [0.30;0.62]	0.02 [0.01;0.10]	0.01 [0.01;0.01]	↓ -1.676	**0.0000**
	Parabacteroides	WATER	0.04 [0.02;0.05]	0.01 [0.01;0.05]	0.03 [0.01;0.08]	-0.062	0.8668
		5-ASA	0.03 [0.02;0.13]	0.06 [0.04;0.09]	0.06 [0.03;0.09]	-0.313	0.3523
		SP	0.03 [0.02;0.04]	0.47 [0.21;0.75]	0.48 [0.23;0.75]	↑ 0.898	**0.0021**
Firmicutes	Anaerostipes	WATER	0.01 [0.01;0.01]	0.02 [0.01;0.03]	0.01 [0.01;0.02]	-0.041	0.7727
		5-ASA	0.01 [0.01;0.01]	0.02 [0.00;0.03]	0.09 [0.00;0.20]	↑ 1.037	**0.0139**
		SP	0.01 [0.01;0.01]	0.04 [0.01;0.07]	0.04 [0.01;0.07]	0.34	0.2500
	Caldicoprobacter	WATER	0.03 [0.02;0.03]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	↓ -1.196	**0.0014**
		5-ASA	0.00 [0.00;0.02]	0.01 [0.01;0.03]	0.01 [0.01;0.01]	0.093	0.8533
		SP	0.02 [0.01;0.03]	0.00 [0.00;0.01]	0.00 [0.00;0.01]	-0.573	0.2120
	Caproiciproducens	WATER	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	-0.264	0.5671
		5-ASA	0.00 [0.00;0.01]	0.00 [0.00;0.02]	0.03 [0.01;0.04]	↑ 0.749	**0.0265**
		SP	0.00 [0.00;0.01]	0.02 [0.01;0.03]	0.02 [0.01;0.03]	0.443	0.0728
	Coprococcus	WATER	0.01 [0.01;0.01]	0.01 [0.00;0.03]	0.01 [0.00;0.03]	0.219	0.5574
		5-ASA	0.01 [0.00;0.01]	0.02 [0.02;0.04]	0.02 [0.02;0.02]	0.336	0.2445
		SP	0.01 [0.01;0.01]	0.38 [0.26;0.42]	0.38 [0.27;0.42]	↑ 1.307	**0.0146**
	Gemella	WATER	0.01 [0.01;0.02]	0.01 [0.00;0.02]	0.00 [0.00;0.01]	↓ -0.666	**0.0463**
		5-ASA	0.00 [0.00;0.01]	0.01 [0.00;0.03]	0.00 [0.00;0.00]	-0.595	0.2332
		SP	0.01 [0.01;0.02]	0.01 [0.00;0.01]	0.01 [0.00;0.01]	-0.228	0.4914
	Lactobacillus	WATER	24.2 [18.7;29.1]	21.2 [16.2;22.3]	22.7 [21.6;24.5]	-0.067	0.5936
		5-ASA	18.4 [17.4;22.6]	33.0 [24.3;40.4]	30.3 [26.9;36.7]	0.133	0.2529
		SP	24.2 [18.7;30.0]	36.5 [32.9;37.9]	38.9 [36.7;42.6]	↑ 0.14	**0.0044**
	Mogibacterium	WATER	0.02 [0.02;0.02]	0.02 [0.01;0.05]	0.05 [0.03;0.08]	0.197	0.4080
		5-ASA	0.01 [0.01;0.02]	0.00 [0.00;0.01]	0.00 [0.00;0.01]	-0.598	0.2561
		SP	0.02 [0.01;0.02]	0.21 [0.11;0.29]	0.23 [0.18;0.29]	↑ 0.588	**0.0196**
	Romboutsia	WATER	7.10 [3.99;9.80]	0.60 [0.17;1.08]	0.83 [0.51;1.08]	-0.963	NaN
		5-ASA	3.59 [2.19;4.85]	4.48 [2.75;6.97]	2.86 [2.24;4.68]	0.036	0.8863
		SP	5.31 [3.99;7.12]	0.64 [0.48;0.77]	0.80 [0.62;1.41]	↓ -0.692	**0.0186**
	Streptococcus	WATER	0.06 [0.05;0.07]	0.02 [0.01;0.03]	0.01 [0.00;0.03]	-0.482	0.1252
		5-ASA	0.07 [0.01;0.19]	0.05 [0.00;0.12]	0.01 [0.00;0.01]	↓ -1.336	**0.0050**
		SP	0.04 [0.01;0.07]	0.02 [0.00;0.05]	0.02 [0.01;0.05]	-0.278	0.3655
	Turicibacter	WATER	0.08 [0.08;0.12]	0.01 [0.00;0.03]	0.01 [0.00;0.03]	↓ -0.798	**0.0057**
		5-ASA	0.08 [0.05;0.12]	0.01 [0.01;0.02]	0.01 [0.01;0.03]	-0.614	0.1796
		SP	0.09 [0.08;0.12]	0.01 [0.00;0.01]	0.01 [0.01;0.01]	↓ -1.324	**0.0002**
Proteobacteria	Haemophilus	WATER	0.03 [0.03;0.05]	0.01 [0.01;0.03]	0.01 [0.01;0.02]	-0.487	0.1286
		5-ASA	0.06 [0.01;0.13]	0.04 [0.00;0.08]	0.00 [0.00;0.00]	↓ -1.211	**0.0194**
		SP	0.02 [0.01;0.05]	0.02 [0.01;0.03]	0.02 [0.01;0.03]	-0.102	0.7862
	Oxalobacter	WATER	0.00 [0.00;0.00]	0.00 [0.00;0.00]	0.00 [0.00;0.00]	NA	NA
		5-ASA	0.00 [0.00;0.00]	0.00 [0.00;0.01]	0.00 [0.00;0.00]	-0.812	0.3824
		SP	0.00 [0.00;0.00]	0.04 [0.03;0.04]	0.04 [0.03;0.04]	↑ 0.663	**0.0297**
	Parasutterella	WATER	0.94 [0.76;0.95]	0.25 [0.18;0.30]	0.32 [0.27;0.38]	↓ -0.412	**0.0260**
		5-ASA	0.52 [0.37;0.70]	0.56 [0.40;0.62]	0.98 [0.60;1.39]	0.304	0.0644
		SP	0.68 [0.37;0.94]	1.52 [0.95;1.90]	1.52 [1.02;1.90]	0.229	0.2059

Fig. 4. Dynamics of microbial changes at the genus level. Color gradients indicate, as follows: green represent increase in the population of bacteria, and red represent a decrease, white does not show significant changes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

various ratios, was totally evaluated as inflammatory cells that accurately reflect inflammatory changes in the tissue depending on localization and severity. Microscopic and histological evaluations were performed by a pathologist using a blind method with respect to rats belonging to a specific group.

Histological and morphometric analysis was performed on a Leica Microsystems specialized computerized hardware complex using a Leica DM 1000 microscope. Data were expressed as arithmetic mean and standard error of the mean.

2.8. DNA sequencing and post-processing

DNAs were isolated from fecal samples using the QIAamp DNA Mini Kit (Qiagen, 51306). The concentration of double-stranded DNA in isolated samples was determined using a Qubit 2.0 instrument and a Qubit dsDNA HS Assay kit (ThermoFisher, catalog number 32853).

Library for Next-generation sequencing (NGS) generated with NEXTflex® 16S V1–V3 Amplicon-Seq Kit (PerkinElmer, catalog number NOVA-4202-04), according to the manufacturer's instructions. 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). Amplicons were sequenced on the MiSeq instrument (Illumina).

Demultiplexing, filtering, denoise, chimeric sequences and determining OTU and taxonomic identification were performed using LotuS pipeline (Hildebrand et al., 2014).

Analysis of alpha diversity to assess the abundance of the community, the calculation of alpha biodiversity (Shannon indice), beta biodiversity as well as the construction of taxonomic distribution at the phylum and genus level were performed using vegan (Oksanen et al., 2020) and phyloseq R packages (v.1.24.2) and graphs were generated using ggplot2 and MicrobiomeAnalyst R packages. Significance testing of longitudinal trends was determined Compound Poisson Linear Models on TSS normalized values using MaAsLin2 package (Mallick et al., 2021) (v. 0.7–7).

2.9. Statistical analysis

Non-parametric Mann-Whitney (MW) and Kruskal-Wallis (KW) tests were used for comparing two or more groups, respectively. The raw read counts were normalized by the total number of reads. A metagenomic biomarker discovery approach, Linear discriminant analysis Effect Size (LEfSe), was used to identify the microbial components whose sequences were statistically different between groups. For LEfSe, Kruskal–Wallis and pairwise Wilcoxon tests were performed, followed by Linear discriminant analysis (LDA) to assess the effect size of each differentially abundant taxon. Bacteria with markedly increased numbers were defined as those with an LDA score (log10) of over 2.

3. Results and discussion

From the selected samples, 425 isolates were isolated and identified. All samples were screened for SCFA's products. The results showed that 86 acetic, lactic, propionic and butyric acids are produced from the selected cultures: *Bifidobacterium longum* 32 cultures, *Bifidobacterium adolescentis* 15 cultures, *Bifidobacterium bifidum* 15 cultures, *Lactobacillus rhamnosus* 7 cultures, *Streptococcus salivarius* 4 cultures, *Bifidobacterium animalis* 3 cultures, *Bifidobacterium pseudocatenulatum* 2 cultures, *Enterococcus durans* 2 cultures, *Lactobacillus ruminis* 2 cultures, *Faecalibacterium prausnitzii* 1 culture, *Eubacterium rectale* 1 culture, *Eubacterium hallii* 1 culture, *Coprococcus eutactus* 1 culture.

Table 1 shows bacteria with a high production of short chain fatty acids. The highest producers are: *Eubacterium hallii* kz2f1, *Faecalibacterium prausnitzii* kz4d9, *Eubacterium rectale* kz4e5, *Enterococcus durans* kz5b9, *Enterococcus durans* kz5b10.

The effect of a biologically active substance based on metabolites of microbial cultures (including fatty acids) and mare's milk was evaluated on a model of ulcerative colitis in rats. The assessment was carried out according to the disease activity index (DAI), which was calculated on a scale from 0 to 4 using the following parameters: weight loss (0, no; 1, 0–5%; 2, 5–10%; 3, 10–20%; 4,> 20%); stool consistency (0: normal; 2: loose stools; 4: watery diarrhea); bleeding (0, no; 1, traces; 2, weak hidden blood; 3, obvious hidden blood; 4, severe bleeding). After 1 week of receiving DSS, the stool was mostly soft, and in some cases pasty, no bleeding was observed. DAI showed no differences with the comparison group taking 5-aminosalicylic acid. The dynamics of the body weight of rats in the experimental groups was relatively the same and did not show any differences with the dynamics of the weight of the control group. To determine the ability of the product to modulate microflora, rat stool samples after 1 week of drug administration. A total of 48 stool samples were taken from 16 male rats. Rat feces samples were collected in sterile centrifuge tubes and immediately frozen at −80 °C.

The total cumulative histomorphometric severity score of colitis is shown in (Table 2).

As Table 2 shows, the histological structure of the intestinal tissue of rat intestines of the healthy control group corresponded to the histological parameters of healthy rats. Histopathological evaluation and microscopy revealed a preserved morphological pattern of the colon mucosa, epithelium, crypt architecture, and submucosa. In the "Water group", pronounced infiltration was observed in all sections of the intestines of each test individual.

In the group of rats treated with SP (Fig. 1), inflammatory infiltration was characterized by increased focal infiltration in the mucosa, and, in terms of histological sections, light mixed infiltrates in its own plate with focal superficial ulceration of the mucosa. The main difference from the control group was the discontinuity of mucosal changes, since in a number of sections infiltrates were observed without defects of the epithelium, violations of the creep architecture were single and were observed only in part of histological sections. The difference in the total histo-morphometric severity score with the control group was statistically significant ($p < 0.05$). Reduced infiltration of granulocytes, macrophages, and lymphocytes into the plate suggests that bacterial culture lysate and mare's milk reduce inflammation in the intestinal wall. The histopathological picture of the large intestine of rats treated with 5-ASA also showed a significant improvement compared with the control group: single inflammatory infiltrates without damage to the epithelium with preserved crypt architecture and evenly located goblet cells.

For the analysis of the microbial community, the V1 – V3 region of the 16S rRNA gene was selected. The depth of coverage was at least 36,700 readings per sample. All sequence sequences were compared with the SILVA database (Quast et al., 2013) using the LotuS bioinformatics process. On average, 66,995 readings per sample were determined. The taxonomic identification of the bacterial community revealed various phyla, of which Firmicutes and Bacteroidetes were the most represented.

To determine the diversity of the intestinal microbial communities of rats in the various studied groups, the α-diversity metric implemented in R was used. The microbial signature of the rat intestine in the model of ulcerative colitis was not fundamentally changed ($p = 0.7$). The use of 10% DSS led to a decrease in biodiversity and α-diversity, at the level of genus, in the experimental groups (Fig. 2). So after creating a model of ulcerative colitis at the genus level, the following taxa decreased: *Ruminococcus*, *Prevotellaceae*_UCG_003, *Treponema, Parabacteroides, Prevotellaceae*_NK3B31_ group, *Monoglobus, Ruminococcus_gauvreauii_* group. Whereas the relative presence of the genera *Negativibacillus* and *Turicibacter* increased. The most significant decrease in biodiversity was observed in the group of rats with DSS-induced colitis without treatment who took water (Fig. 3D). In the group taking the biological product based on metabolites of microbial cultures, the biodiversity of intestinal microflora increased after 21 days of administration. While the Shannon index in the group receiving 5-ASA (5-aminosalicylic acid) was comparable to the group of healthy animals. Decreased intestinal microflora

biodiversity is associated with ulceration (caused by DSS) and local activation of neutrophils and macrophages. PCoA analysis demonstrated a significant difference in microflora with multidimensional scaling. Fig. 3E shows a significant difference in biodiversity between the groups receiving the biological product (red ellipse) and 5-aminosalicylic acid (yellow ellipse).

Determination of the effect of the treatment method on the number of bacterial taxa showed that in the group of animals without treatment (taking water), only the genus *Collinsella* significantly increased ($p = 0.0355$), in contrast, there was a decrease in *Caldicoprobacter, Gemella, Turicibacter, Parasutterella.* In the group treated with 5-ASA significantly increased *Anaerostipes* ($p = 0.0139$), *Coproiciproducens* ($p = 0.0265$), decreased such as the genera *Rothia, Streptococcus, Haemophilus*. The use of metabolites of lactic acid microorganisms in conjunction with mare's milk showed an increase in the relative amount of *Parabacteroides* ($p = 0.0021$), *Coprococcus* ($p = 0.0146$) after the first week of use and remained at the same level after three weeks of administration. The *Lactobacillus* taxon increased with SP administration, up to 21 days of intake ($p = 0.0044$). Such an observation can be explained by the stimulating ability of mare's milk and metabolites to stimulate their growth. The similar results have been reported by Xia et al. Shown that the use of probiotic bacteria *Lactobacillus plantarum* AR113 significantly increased the presence of taxa *Lactobacillus* and *Lachnospiraceae* (Xia et al., 2020). An increase in the genera *Mogibacterium, Oxalobacter* was also observed (Fig. 4).

In this study, the following bacterial genera were included into the group of healthy animals of the top 10 taxa: *Lactobacillus, Prevotella, Romboutsia, Lachnospiraceae, Mycoplasma, Rikenella, Parabacteroides, Bacteroides, Parasutterella,* and *Ruminococcus*. After the formation of a model of DSS-induced colitis, *Treponema, Butyricicoccus,* and *Turicibacter* entered the top 10 taxa. At the same time, the number of mycoplasmas, rickenella, parabacteroids decreased. Results match previously published data (Gao et al., 2018). The abundance of *Escherichia* and *Bacteroides* increased significantly in DSS group same as in research (Wang et al., 2019).

Our findings indicate that the use of a biological product based on bacterial metabolites (including fatty acids) led to a shift in the bacterial structure of the intestinal microbiome with an increase in gram-positive *Lactobacillus*, *Coprococcus, Mogibacterium,* and gram-negative *Parabacteroides, Oxalobacter.*

4. Conclusions

In fact, a shift in microbiota can directly affect the vital functions of the organism (Järbrink-Sehgal & Andreasson, 2020; Wang, Xiong, et al., 2020, p. 53). In our study, we observed an increase in SCFAs producing strains, including *Lactobacillus* as a result of the application of a biological product. In addition, a decrease in the number of *Romboutsia, Turicibacter* and other representatives of the pathogenic intestinal microflora was detected as well. Our results indicated that the biologically active substances of bacteria and mare's milk possess therapeutic potential for the treatment of ulcerative colitis. However, additional studies are needed to confirm its effectiveness and clinical relevance.

Author statement

SK, DB, SK, and AK: conceptualization and data curation. SK, DB, and SK: formal analysis and visualization. SK: funding acquisition. MN, NM, AN, LC, SS, AG, and BT: methodology. SK, AK, and TM: project administration, resources, and supervision. SK and DB: writing – original draft. All authors: writing – review and editing.

Declaration of competing interest

The authors have no financial conflicts of interest to declare.

Acknowledgments

The authors thankful to the Ministry of Agriculture of the Republic of Kazakhstan for funding this research work:Development of technologies using new strains of beneficial microorganisms, enzymes, nutrients, and other kits in the production of special dietary food products IRN: BR10764998; Ministry of Education and Science Republic of Kazakhstan for grant: IRN AP05134659; Nazarbayev University for grant: MEPD:021220CRP0622.

References

Abboud, M., & Papandreou, D. (2019). Gut microbiome, probiotics and bone: An updated Mini review. *Open Access Macedonian Journal of Medical Sciences, 7*(3), 478–481. https://doi.org/10.3889/oamjms.2019.047

Ahn, J., Sinha, R., Pei, Z., Dominianni, C., Wu, J., Shi, J., Goedert, J. J., Hayes, R. B., & Yang, L. (2013). Human gut microbiome and risk for colorectal cancer. *Journal of the National Cancer Institute, 105*(24), 1907–1911. https://doi.org/10.1093/jnci/djt300

Chassaing, B., Aitken, J. D., Malleshappa, M., & Vijay-Kumar, M. (2014). Dextran sulfate sodium (DSS)-induced colitis in mice. *Current Protocols in Immunology, 104*, 15–25. https://doi.org/10.1002/0471142735.im1525s104, 1-15.25.14.

Council of Europe. (2006). European convention for the protection of vertebrate animals used for experimental and other scientific Purposes (ETS No. 123). Guidelines for accommodation and care of animals (article 5 of the convention). Approved by the multila. *Cons, 123*(3).

Gao, Z., Chen, K.-Y., Mueller, O., Zhang, H., Rakhilin, N., Chen, J., & Shen, X. (2018). In *Microbiota of inflammatory bowel disease models. Conference Proceedings : Annual international conference of the IEEE engineering in medicine and biology society*. IEEE Engineering in Medicine and Biology Society. https://doi.org/10.1109/EMBC.2018.8512848. Annual Conference, *2018*, 2374–2377.

Hewett, T. A., Kovacs, M. S., Artwohl, J. E., & Bennett, B. T. (1993). A comparison of euthanasia methods in rats, using carbon dioxide in prefilled and fixed flow rate filled chambers. *Laboratory Animal Science, 43*(6), 579–582.

Hildebrand, F., Tadeo, R., Voigt, A. Y., Bork, P., & Raes, J. (2014). LotuS: An efficient and user-friendly OTU processing pipeline. *Microbiome, 2*(1), 30. https://doi.org/10.1186/2049-2618-2-30

Järbrink-Sehgal, E., & Andreasson, A. (2020). The gut microbiota and mental health in adults. *Current Opinion in Neurobiology, 62*, 102–114. https://doi.org/10.1016/j.conb.2020.01.016

Karlsson, F., Tremaroli, V., Nielsen, J., & Bäckhed, F. (2013). Assessing the human gut microbiota in metabolic diseases. *Diabetes, 62*(10), 3341–3349. https://doi.org/10.2337/db13-0844

Koelink, P. J., Wildenberg, M. E., Stitt, L. W., Feagan, B. G., Koldijk, M., van 't Wout, A. B., Atreya, R., Vieth, M., Brandse, J. F., Duijst, S., Te Velde, A. A., D'Haens, G. R. A. M., Levesque, B. G., & van den Brink, G. R. (2018). Development of reliable, valid and responsive scoring systems for endoscopy and histology in animal models for inflammatory bowel disease. *Journal of Crohn's & Colitis, 12*(7), 794–803. https://doi.org/10.1093/ecco-jcc/jjy035

Lapthorne, S., Pereira-Fantini, P. M., Fouhy, F., Wilson, G., Thomas, S. L., Dellios, N. L., Scurr, M., O'Sullivan, O., Ross, R. P., Stanton, C., Fitzgerald, G. F., Cotter, P. D., & Bines, J. E. (2013). Gut microbial diversity is reduced and is associated with colonic inflammation in a piglet model of short bowel syndrome. *Gut Microbes, 4*(3), 212–221. https://doi.org/10.4161/gmic.24372

Macfarlane, S., & Macfarlane, G. T. (2003). Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society, 62*(1), 67–72. https://doi.org/10.1079/PNS2002207

Mallick, H., Rahnavard, A., McIver, L., Ma, S., Zhang, Y., Nguyen, L., Tickle, T., Weingart, G., Ren, B., Schwager, E., Chatterjee, S., Thompson, K., Wilkinson, J., Subramanian, A., Lu, Y., Waldron, L., Paulson, J., Franzosa, E., Corrada Bravo, H., & Huttenhower, C. (2021). *Multivariable association discovery in population-scale meta-omics studies.* https://doi.org/10.1101/2021.01.20.427420

Morrison, D. J., & Preston, T. (2016). Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes, 7*(3), 189–200. https://doi.org/10.1080/19490976.2015.1134082

National Research Council. (2011). *Guide for the Care and use of laboratory animals* (8th ed.). The National Academies Press. https://doi.org/10.17226/12910

Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., Hara, R. B. O., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Szoecs, E. (2020). *Package ' vegan. '*.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research, 41*, D590–D596. https://doi.org/10.1093/nar/gks1219. Database issue.

Roy, C. C., Kien, C. L., Bouthillier, L., & Levy, E. (2006). Short-chain fatty acids: Ready for prime time? *Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition, 21*(4), 351–366. https://doi.org/10.1177/0115426506021004351

Schulthess, B., Bloemberg, G. V., Zbinden, R., Bottger, E. C., & Hombach, M. (2014). Evaluation of the bruker MALDI biotyper for identification of gram-positive rods: Development of a diagnostic algorithm for the clinical laboratory. *Journal of Clinical Microbiology, 52*(4), 1089–1097. https://doi.org/10.1128/JCM.02399-13

Shen, Z.-H., Zhu, C.-X., Quan, Y.-S., Yang, Z.-Y., Wu, S., Luo, W.-W., Tan, B., & Wang, X.-Y. (2018). Relationship between intestinal microbiota and ulcerative colitis: Mechanisms and clinical application of probiotics and fecal microbiota transplantation. *World Journal of Gastroenterology, 24*(1), 5–14. https://doi.org/10.3748/wjg.v24.i1.5

Wang, J., Xiong, K., Zhao, S., Zhang, C., Zhang, J., Xu, L., & Ma, A. (2020). Long-term effects of multi-drug-resistant tuberculosis treatment on gut microbiota and its health consequences. In *Frontiers in microbiology* (Vol. 11). p. 53 https://www.frontiersin.org/article/10.3389/fmicb.2020.00053.

Wang, W., Xing, W., Wei, S., Gao, Q., Wei, X., Shi, L., Kong, Y., & Su, Z. (2019). Semi-rational screening of probiotics from the fecal flora of healthy adults against DSS-induced colitis mice by enhancing anti-inflammatory activity and modulating the gut microbiota. *Journal of Microbiology and Biotechnology, 29*(9), 1478–1487. https://doi.org/10.4014/jmb.1807.06061

Wang, Y., Ouyang, M., Gao, X., Wang, S., Fu, C., Zeng, J., & He, X. (2020). Phocea, pseudoflavonifractor and Lactobacillus intestinalis: Three potential biomarkers of gut microbiota that affect progression and complications of obesity-induced type 2 diabetes mellitus. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 13*, 835–850. https://doi.org/10.2147/DMSO.S240728

Weinstein, M. P., Patel, J. B., Campeau, S., Eliopoulos, G. M., Galas, M. F., Humphries, R. M., Jenkins, S. G., II, L, J. S., Limbago, B., Mathers, A. J., Mazzulli, T., Patel, R., Richter, S. S., Satlin, M., Swenson, J. M., & Zimmer, B. L. (2018). *Clinical Microbiology Procedures Handbook*. http://www.facm.ucl.ac.be/intranet/CLSI/CLSI-2018-M100-S28-unlocked.pdf.

Xia, Y., Chen, Y., Wang, G., Yang, Y., Song, X., Xiong, Z., Zhang, H., Lai, P., Wang, S., & Ai, L. (2020). Lactobacillus plantarum AR113 alleviates DSS-induced colitis by regulating the TLR4/MyD88/NF-κB pathway and gut microbiota composition. *Journal of Functional Foods, 67*, 103854. https://doi.org/10.1016/j.jff.2020.103854

Yoshida, N., Yamashita, T., & Hirata, K.-I. (2018). Gut microbiome and cardiovascular diseases. *Diseases, 6*(3), 56. https://doi.org/10.3390/diseases6030056