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Modulatory effects of *Lactococcus lactis* on gut microbiota of albino rats induced with *Escherichia coli* O157:H7

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ABSTRACT

Background: The human microbiome, comprises of diverse microbial communities, is crucial for maintaining human health, especially within the gut. *Escherichia coli* O157:H7, known for its Shiga toxin production, is a major cause of diarrhea. Lactic acid bacteria (LAB) are recognized for their potential in preventing *E. coli* O157:H7 infection. This study aimed to assess the impact of LAB treatment on the intestinal microbiota of albino rats infected with *E. coli* O157:H7. **Methods:** Experimental albino rats were induced with *Escherichia coli* and diarrhea set in after three days and treated with *Lactococcus lactis* as probiotics, compared to a control group. Stool samples underwent 16S metagenomic profiling using the Pacbio platform. **Results:** it showed a bacterial dominance in the Kingdom domain, with increased read counts post-treatment. Phylum-level analysis revealed dominance by *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Cyanobacteria*, and *Bacteroidetes*. Approximately half of the identified taxa were novel "Phylotypes." Predominant species/phylotypes included *Pediococcus acidilactici*, *Pediococcus*, *Collinsella aerofaciens*, and *Lactobacillus*. While some organisms decreased post-treatment, new commensals and symbionts were acquired. **Conclusion:** this study highlights significant differences in the intestinal microbiota of albino rats pre- and post-LAB treatment, indicating *L. lactis*' efficacy in alleviating *E. coli* O157:H7-induced diarrhea and enriching gut microbiota.

Introduction

The gut microbiota, comprised of bacteria residing in the gastrointestinal tract, plays a crucial role in host physiology. In mammals, these resident microbes enhance digestion efficiency by synthesizing vitamins, breaking down fiber, and providing fermented energy [1]. Recent studies have highlighted their involvement in detoxification, immune system maturation, behavior, and post-embryonic development, influencing various

aspects of host physiology, ecology, and evolution [2]. Probiotics have been recognized for their impact on gut microbiota diversity in animals [3].

Imbalances in the gut microbiota composition can lead to diseases, with inflammatory bowel disease being linked to gut bacteria interactions. Clinical studies suggest that enhancing gut microbiota diversity can benefit inflammatory bowel disease treatment [1]. Despite the benefits of

commensal microbial communities, maintaining a balanced microbiome is crucial for protection against pathogens [4]. Disruptions in this balance can lead to illness [5], highlighting the importance of a healthy gut flora. Commensal bacteria, both beneficial and harmful, regulate host cell proliferation and tissue healing [6], emphasizing the role of probiotics in preventing pathogen growth and maintaining gut barrier integrity.

The gut microbiota significantly influences mucosal immune system development, impacting its structure and function [7]. Diarrheal diseases pose a significant global health burden, especially in children under five. According to the World Health Organization, diarrhea is the second leading cause of death in this age group, claiming over 760,000 lives annually and contributing to childhood malnutrition and stunting [8]. Despite a decrease in diarrheal deaths over the past three decades, efforts to further reduce mortality remain imperative [9].

In northern Nigeria and neighboring Chad and Niger, "Kunun-zaki," a fermented non-alcoholic cereal beverage, is popular for its sweet-sour flavor, typical of lactic acid bacterial fermented foods [10, 11]. It encompasses various non-alcoholic cereal-based beverages, differentiated by the base cereal grain [12-14]. The fermentation process involves a diverse range of microorganisms, including Lactic Acid Bacteria (LAB) such as *Lactococcus lactis*, the probiotic used in this study to treat diarrhea in albino rats. However, only select LAB strains determine the quality of the final product.

The gut microbiota plays a pivotal role in maintaining host health, influencing various physiological processes, including digestion, immune function, and protection against pathogens. Disruptions in the gut microbiota, often termed dysbiosis, have been linked to a wide range of diseases, including inflammatory bowel disease, obesity, and infections caused by pathogenic bacteria such as *Escherichia coli* O157:H7 [15]. *E. coli* O157:H7 is a significant foodborne pathogen known for causing severe gastrointestinal illnesses, which can lead to serious complications like hemolytic uremic syndrome (HUS). Therefore, strategies to mitigate the adverse effects of such pathogens are of considerable interest in both clinical and food safety contexts [16].

Probiotics, particularly those from the genus *Lactococcus*, have garnered attention for their potential to restore and maintain gut microbiota

balance. *Lactococcus lactis*, a lactic acid bacterium widely used in the dairy industry, has shown promising probiotic properties, including the ability to inhibit pathogenic bacteria, modulate the immune response, and enhance gut barrier function [17]. However, there is limited research on the specific modulatory effects of *Lactococcus lactis* on gut microbiota in the presence of pathogenic *E. coli* strains.

This study aims to investigate the modulatory effects of *Lactococcus lactis* on the gut microbiota of albino rats induced with *E. coli* O157:H7. By exploring the interactions between *Lactococcus lactis* and the gut microbiota under pathogenic stress, this research seeks to elucidate the potential of *Lactococcus lactis* as a biotherapeutic agent. We hypothesize that the administration of *Lactococcus lactis* will not only reduce the colonization of *E. coli* O157:H7 in the gut but also promote a beneficial shift in the gut microbial community, thereby enhancing overall gut health.

Understanding these interactions is crucial for developing effective probiotic interventions that can mitigate the adverse effects of pathogenic infections and contribute to the maintenance of a healthy gut microbiome. This study provides a foundation for further exploration into the application of *Lactococcus lactis* in managing gut health and combating foodborne pathogens.

Materials and methods

Source of microorganism (bacteria species)

The native fermented beverage "Kunun zaki," which is popular in northern Nigeria, is the source of the *Lactococcus lactis*. They were cultivated for 24 hours at 37°C in de Man-Ragosa Sharp broth. The probiotics had a concentration of about 10⁹ CFU/ml. *Escherichia coli* O157:H7 was obtained from the isolate bank at the molecular microbiology lab at the University of Jos. The organisms were kept alive for 24 hours at 37°C in nutrient broth, where their concentration was 10⁶ CFU/ml. All of the bacteria were kept in storage at 40°C until being used.

Animal care and experimental groups

Ethical certification reference number, UJ/FPS/F17-00379 was issued by the Ethical Committee Animal Experimental Unit of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. Five weeks old pathogen free albino rats weighing 85-105g were purchased from the Faculty of

Pharmaceutical Science, University of Jos, where ethical clearance was duly obtained. The rats were acclimatized for one week; they were housed in metabolic cages at suitable temperature and humidity and were fed appropriately, accompanied with water *ad libitum*. The animals were then divided into two experimental groups (n=5); Test group (T₁), and the negative control group.

The rats were administered an *Escherichia coli* species that causes diarrhea (*Escherichia coli* O157:H7) after one week of adapted feeding. The administration process involved oral gavage, a common method for delivering precise doses of substances directly to the stomach of laboratory animals [18]. The dosage of *E. coli* O157:H7 was standardized based on established protocols in microbial pathogenicity studies [19]. Specifically, the rats received a suspension containing approximately 10⁸ colony-forming units (CFU) of *E. coli* O157:H7 per milliliter [20]. This dosage was chosen in accordance with the standards set by the National Institutes of Health (NIH) guidelines for bacterial infection models in rodents, ensuring a consistent and replicable induction of gastrointestinal infection [21]. In northern Nigeria, a fermented drink called "Kunun zaki" that is derived from guinea corn is where *Lactococcus lactis* was found. The specific species employed in the study were cultivated in de Man-Ragosa Sharpe broth for 24 hours at 37°C, with a probiotic concentration of roughly 10⁹ CFU/ml.

Animal treatment

After one week of adaptive feeding, the rats were induced with *Escherichia coli* and diarrhea set in after three days. They were then administered *L. lactis* for five days as treatments. The other subjects were infected with *Escherichia coli* for three days, but had no Lactic acid bacteria (LAB) intervention until they were sacrificed.

Collection of fecal samples, DNA extraction and PCR amplification

Following anesthesia, cervical dislocation was used to kill the albino rats. Fresh feces samples from the mice were taken on the final day of the experiment, put in sterile containers, and frozen at -

80°C for later study [7]. The preserved stool samples were sent to Inqaba Biotec, Africa's Genomic Company, South Africa. Metagenomic analysis of full length 16S gene amplicons. Samples were sequenced on the sequel system by PacBio (www.pacb.com). Raw subreads were processed through the SMRTlink (v10.2) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). These highly accurate reads were processed through DADA2 (<https://benjjneb.github.io/dada2/index.html>) and qiime2 (<https://docs.qiime2.org/2021.11/>) for quality control assessment and taxonomic classification.

Standard Primers from Inqaba Biotechnical Industries, South Africa:

27-F: /5AmMC6/gtaaacgacggccagt
AGRGTTYGATYMTGGCTCAG

1492-R: /5AmMC6/caggaacagctatgac
RGYTACCTTGTTACGACTT

Statistical analysis

Operational Taxonomic Units (OTUs) are phylogenetic or population genetic studies that pinpoint the level of taxonomic classification (phylum, genus, species, group, etc.) similarities between closely related individuals. In general, 97% similarity is used for statistical analysis of biological data. To facilitate group comparison, all sequences were OTU partitioned using the Usearch software platform (version 7.1; available <http://drive5.com/uparse/>). Simple Pie charts were used to present results.

Results

The taxonomic classification of the stool samples obtained from albino rats challenged with *Escherichia coli* O157:H and treated with *Lactococcus lactis*.

The Kingdom classification is 100% bacteria (with read count of 23242.0).

The phylum classification is 94% *Firmicutes* before (B1) and after (A1) the treatment with *Lactococcus lactis*.

Table 1. (B1) Before the Treatment with *Lactococcus lactis*

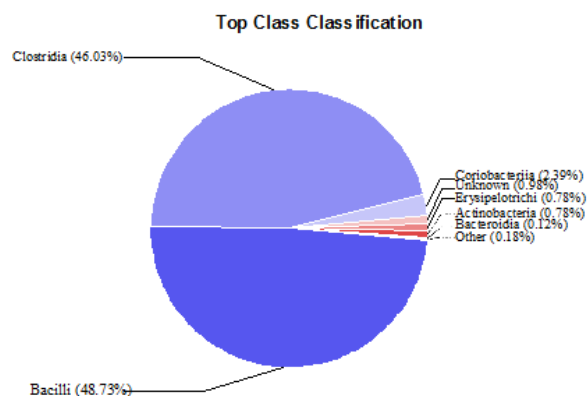
Phyla	Read Count	%
Firmicutes	18217.0	94.43
Actinobacteria	636.0	3.30
Unknown	267.0	1.38
Unknown	56.0	0.29
Bacteroidetes	36.0	0.19
Proteobacteria	26.0	0.13
TM7	25.0	0.13
Tenericutes	15.0	0.08
Cyanobacteria	12.0	0.06
Planctomycetes	2.0	0.01

Table 2. (A1) After Treatment with *Lactococcus lactis*

	Read Count	%
Firmicutes	22321.0	96.05
Actinobacteria	737.0	3.17
Unknown	110.0	0.47
Bacteroidetes	29.0	0.12
Unknown	24.0	0.10
Proteobacteria	12.0	0.05
Cyanobacteria	6.0	0.03

Class classification

Figure 1. The top-class classification is seen to host Bacilli (36.93%), *Clostridium* (51.14%) and others. No missing organisms nor new organisms observed.



Order classification

Figure 2. Order classification before treatment with *Lactococcus lactis* (B1). The order classification *Lactobacillales* (35.39%) and *Clostridiales* (51.14%) top the order classification.

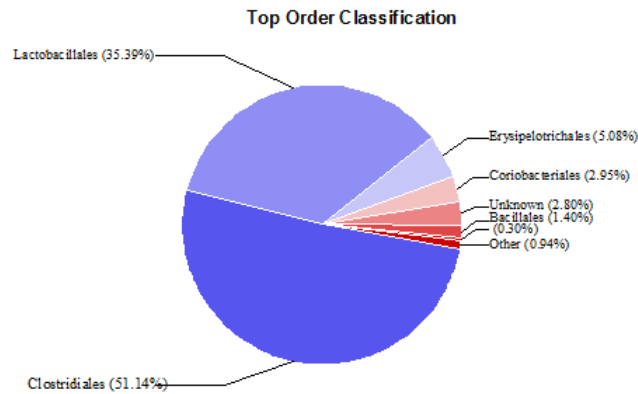
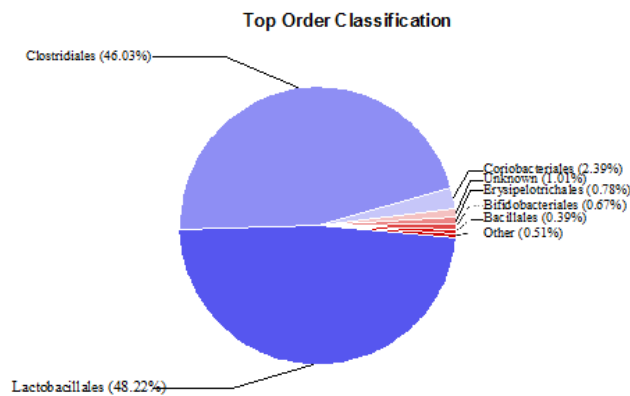


Figure 3. Order classification (A1) after treatment with *Lactococcus lactis*. Note that after the treatment the following organisms were missing in the group: *Desulfovibrionales*, *Pirellulales*, CW040 and RF39. No new organism was seen after the treatment at the order level but changes in percentage increase or decrease were observed with the following organisms: *Lactobacillales* (12, 83%) and *Clostridiales* (4.97%).



Family classification

Figure 4. Top family classification for B1 had *Peptostreptococcaceae* and *Lactobacillaceae* top the group in its percentage appearance.

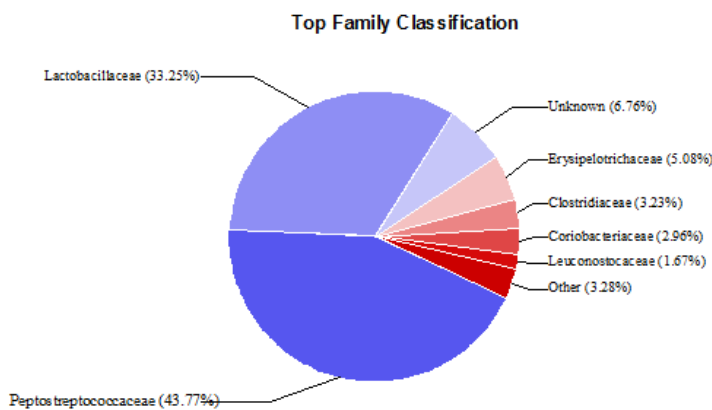
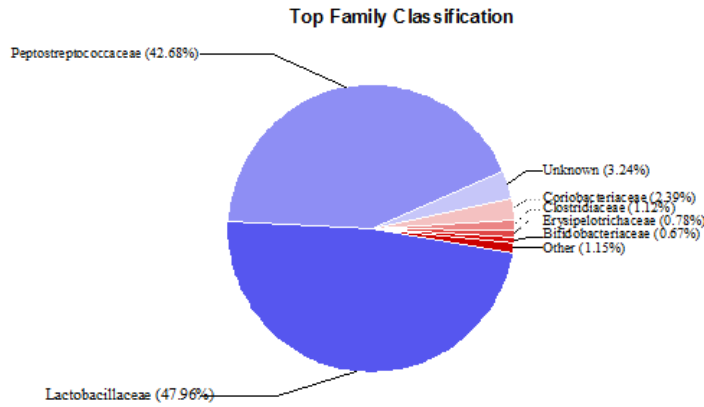


Figure 5. Family level of classification (A1). The following organisms were missing after treatment with *L. lactis*: *Staphylococcaceae*, *Desulfovibrionaceae*, *Brevibacteriaceae*, *Micrococcaceae*, *Comamonadaceae*, *Porphyromonadaceae*, *Pirellulaceae*, *S24* and *F16*. Two new organisms were found; *Streptomycetaceae* and *Eubacteriaceae*. *Lactobacillaceae* had 14.68% increase in abundance.



Top genus classification

Figure 6. Top genus classification (B1) showing *Pediococcus* (46.28%) and Unknown species (47.96%). Eight organisms present in the group (*Oscillospira*, *Lawsonia*, *Brevibacterium*, *Desulfovibrio*, *Rothia*, *Lactococcus*, *Staphylococcus* and *Collinsella*), unlike the treated group below.

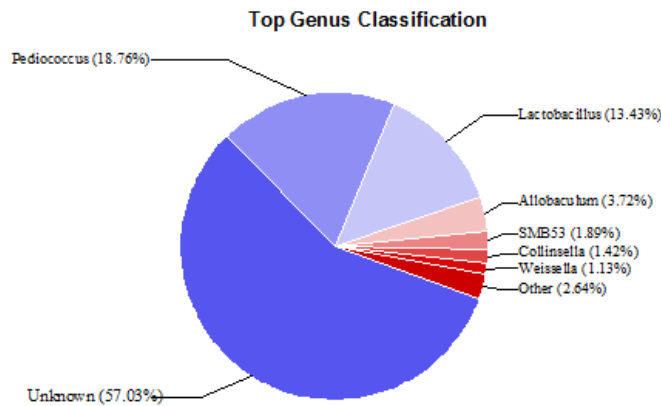
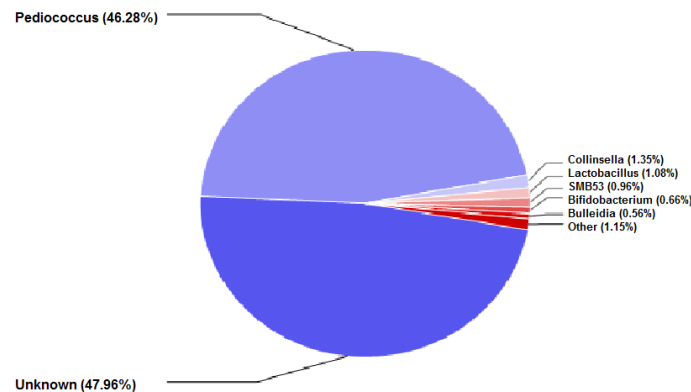


Figure7. Top genus classification (A1). The eight organisms present in the group before treatment were observed to be missing after the treatment. Moreover, the following new organisms were observed after the treatment; *Fingoldia*, *Veillonella*, *Anaerococcus*, *Blautia*, *Weissella*, *Dialister*, *Anaerofustis*, and *Streptomyces* out of which three organisms *Pediococcus* (28.48%), *Lactobacillus* (12.35%) and Unknown (9.93%) were significant in concentration.



Species level of classification

Figure 8. Species level of classification (B1), *Pedococcus acidilactici* and *Lactobaccillus* topped the group with the percentage abundance of 13.80% and 13.43% respectively before the treatment.

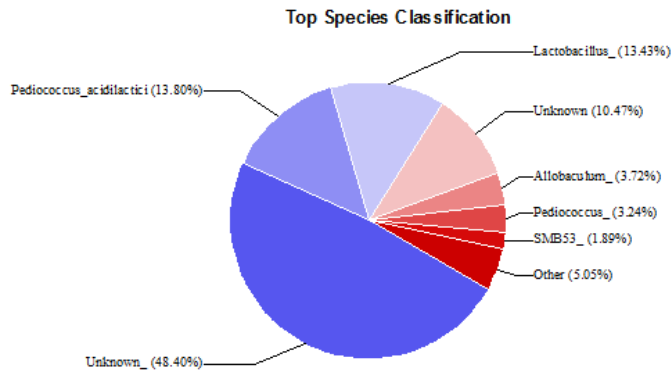
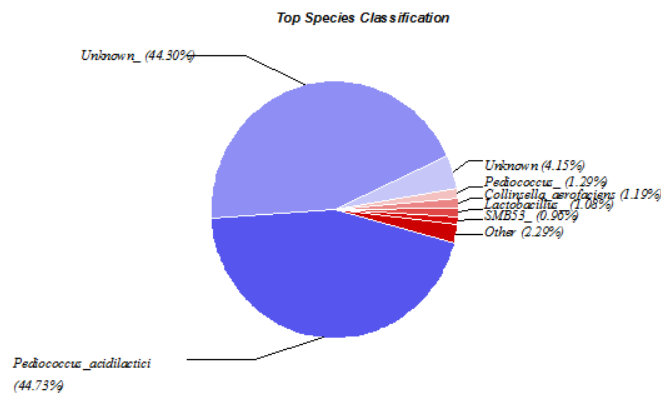


Figure 9. The species level of classification A1. After the treatment, we found that *Pedococcus acidilactici* only top the list with 44.73% percentage increase in abundance where *Lactobaccillus* (1.08%) show a drastic decrease in percentage abundance. Fourteen organisms disappeared after the treatment; *Weissella viridescens*, *Stapylococcus*, *Staphylococcus saprophyticus*, *Clostridium perfringens*, *Weissella hellenica*, *Oscillospira*, *Unknown gnavus*, *Lawsonia intracellularis*, *Corynebacterium*, *Desulfovibrio*, *Brevibacterium aureum*, *Staphylococcus sciuri*, *Actinomyces* and *Rothia nasirmurium*. However, eleven new organisms evolved after the treatment and the new organisms includes *Bifidobacterium longum*, *Finegoldia*, *Collinsella stercoris*, *Clostridium xylanolyticum*, *Blautia product*, *Dialister*, *Escherichia coli*, *Actinomyces*, *Anaerofustis*, *Veillonella dispar* and *Streptomyces*. Meanwhile, it was observed that *Pedococcus acidilactici* had 30.5% increase in percentage count. There were significant changes in percentage count for all the organisms across the levels of classification, when compared with the before (B1) treatment and after (A1) treatment.



Discussion

Gut flora promotes health, and interference with them is known to cause gastro-intestinal, auto-immune and atopic diseases. Probiotics therefore alleviate gut flora-related illnesses [22]. The present study showed the modulatory effects of *Lactococcus lactis* when used as treatment against *E. coli* O157:H7 infection. One of the key components of a balanced gut microbiome is thought to be the variety and quantity of bacterial species. Healthy gut flora depends on a community-wide equilibrium, and changes to this balance can result in disease [7].

Commensal bacteria play a role in both digestive system health and disease. The characteristics of the intestinal microbiota in albino rats treated with lactic acid bacteria (LAB) and infected with *E. coli* O157:H7 were examined in this work before and after the intervention, and OTU values were determined using 16S metagenomic sequencing.

It was observed in this study that the outcomes between the groups (B1) and (A1) before and after treatment with probiotics were greatly different. This is in line with the research results from [23] on the inhibitory effects of *Lactobaccillus*

acidophilus and *Lactobacillus casei* isolated from "Kunun zaki" (A Nigerian Fermented Beverage) against *Helicobacter pylori*. Probiotics are defined as live microorganisms that, when administered in sufficient amounts, have a positive effect on the host's microbiota and benefit the host [13]. Fermentable LAB are aerobic, prevalent in the environment and diet, and rule the small intestine's symbiotic flora [24]. Therefore, treatment with LAB can effectively prevent the structural disruption of the gut microbiota and protect the intestinal tract from pathogens as discussed by [25]. The two most prevalent taxa in the current investigation were Firmicutes, Actinobacteria, and Bacteroidetes. According to research by [26], bacteria from the three major taxa Firmicutes, Bacteroidetes, and Actinobacteria make up the majority of the varied gut microbiota. In the gut microbiota, glutathione, a natural antioxidant, can be stimulated by the dominant phylum of firmicutes. It also maintains a healthy gut lining and reduces intestinal inflammation, which inhibits the growth of cancer cells [27]. Species like *Pedicoccus acidilactici*, *Lactobacillus*, *Bifidobacterium* species, *Streptococcus* species, *Ruminococcus* etc. were some of the beneficial gut bacteria that gained an increase in number after the treatment with LAB. Species like *Pedicoccus acidilactici*, had a significant increase of 31.13% after the treatment with LAB making it the richest species observed. This supports the findings of [3], which demonstrated that *Pedicoccus acidilactici* is a healthy bacteria used as starter cultures, and some strains can produce bacteriocins and other antimicrobial metabolites such as pediocins. As probiotic, it enriches the gut by balancing the intestinal flora, promoting healthy inflammatory response and immune response [28]. Probiotic properties of *Pedicocci* have been successful for humans and other animals with the aim of immunomodulation, control of pathogens, improved recovery after COVID-19, antibiotic therapy and improved health status (reduction of the incidence of respiratory tract infections and the management of lactose intolerance) [29]. Studies have shown that the proportion of the intestinal microbiota differs between health individuals and those who are not [7]. The result of this study is in line with afore mentioned, that the structure of the intestinal flora of albino rats treated with LAB was restored and the diarrhea caused by of *E. coli* O157:H7 infection

improved [30], indicating that intestinal health is associated with disease recovery.

Conclusion

This study reveals that *L. lactis* has therapeutic effects against *E. coli* O157:H7 and have shown remarkable abilities for controlling diarrhea and also enriched and improved the gut. The administration of LAB as a probiotic has shown to enhance and enrich the gut microbiota following diarrheal treatment. The beneficial effects of LAB extend beyond pathogen eradication, contributing to the restoration and improvement of the overall composition and diversity of the gut microbial community.

Recommendation

Based on the results of the current study, lactic acid bacteria (*Lactococcus lactis*) from kunun-zaki had shown a notable difference in the composition and diversity of the intestinal microbiota of the albino rats before their treatment and after their treatment, thus *L. lactis* cured *E. coli* O157:H7-induced diarrhea and also significantly enriched gut microbiota, suggesting that they could be potential probiotics for inclusion in the fermentation of beverages.

Data Availability

The accession number from GeneBank-OR518626

Conflict of interest

The authors declare no conflict of interest.

Fund statement

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