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EVALUATION OF THE ANTIULCER AND ANTIDIARRHEAL POTENTIAL OF LACTIC ACID BACTERIA ISOLATED FROM YOGURT IN ULCER AND DIARRHEA INDUCED IN WISTAR RATS

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ABSTRACT

This study investigates the antiulcer and antidiarrheal potential of lactic acid bacteria (LAB) isolated from commercially available yogurt in Wistar rats. Three LAB strains, Lactobacillus paracasei (Y1), Lactobacillus rhamnosus (Y2), and Streptococcus thermophilus (Y3), were identified through biochemical tests and 16S rRNA sequencing. For the antiulcer study, rats were pre-treated with LAB strains for seven days before inducing gastric ulcers with indomethacin. Ulcer index and gastric juice parameters were assessed. For the antidiarrheal study, diarrhea was induced using castor oil, and the effects of LAB pre-treatment on fecal output, consistency, and intestinal transit time were evaluated. GC-MS analysis identified bioactive compounds that may contribute to the observed therapeutic effects. Results showed that L. paracasei (Y1) significantly reduced ulcer index by 65% ($p<0.05$) and increased gastric mucus production by 40% compared to the control group. L. rhamnosus (Y2) reduced diarrhea severity by 50% ($p<0.05$) and normalized intestinal transit time. S. thermophilus (Y3) showed moderate improvement in both ulcer and diarrhea parameters. Bioactive compounds such as lactic acid, acetic acid, butyric acid, and reuterin were identified, which may contribute to the protective effects. These findings suggest that LAB isolated from yogurt possess significant antiulcer and antidiarrheal properties, with L. paracasei and L. rhamnosus exhibiting the most promising effects.

Keywords: Lactic acid bacteria, yogurt, antiulcer, antidiarrheal, Wistar rats, probiotics, bioactive compounds, GC-MS

INTRODUCTION

Gastrointestinal disorders, including gastric ulcers and diarrhea, are significant global health concerns, affecting millions worldwide. Gastric ulcers result from the erosion of the stomach lining, often caused by Helicobacter pylori infection, non-steroidal anti-inflammatory drugs (NSAIDs), excessive alcohol consumption, and chronic stress **(Kusters, van Vliet, & Kuipers, 2020; Zakari et al., 2025)**. These ulcers can lead to severe complications such as bleeding and perforation. Current treatment strategies include proton pump inhibitors (PPIs), H2-receptor antagonists, and antibiotics; however, these have limitations, including drug resistance and adverse effects **(Shi et al., 2020)**.

Diarrhea, characterized by frequent, loose, or watery stools, is another prevalent gastrointestinal disorder. It is caused by infections, inflammatory conditions, or dysbiosis of the gut microbiota **(Moura et al., 2020)**. Castor oil-induced diarrhea models demonstrate how intestinal motility and secretion changes contribute to diarrhea **(Zakari et al., 2024)**. While conventional antidiarrheal drugs are available, they often cause constipation and fail to address the underlying gut microbial imbalance **(De Wolfe et al., 2021)**.

Probiotics, particularly lactic acid bacteria (LAB), are gaining attention as natural therapeutic agents for gastrointestinal disorders. LAB strains from yogurt have demonstrated potential in modulating gut microbiota, strengthening mucosal barriers, and exerting antimicrobial effects **(Markowiak & Śliżewska, 2021)**. However, their specific bioactive components and mechanisms of action require further investigation. This study evaluates the antiulcer and antidiarrheal properties of LAB isolated from yogurt in an animal model and identifies the bioactive compounds responsible using GC-MS.

Lactic acid bacteria (LAB) are a group of beneficial microorganisms commonly found in fermented foods like yogurt. They have demonstrated potential health benefits, including the ability to modulate the gut microbiota, enhance immune function, and produce antimicrobial substances **(Ouwehand, Tiihonen, Vesikari, Rautonen, & Salminen, 2021)**. Certain LAB strains have shown promise in preventing and treating gastric ulcers and diarrhea in animal models and clinical trials **(Gao et al., 2021)**. Their mechanisms of action are multifaceted and may involve reducing inflammation, strengthening the gut barrier, and inhibiting the growth of pathogenic bacteria.

This study aimed to evaluate the antiulcer and antidiarrheal potential of LAB isolated from commercially available yogurt in Wistar rats. We hypothesized that pre-treatment with selected LAB strains would ameliorate the severity of experimentally induced gastric ulcers and diarrhea.

MATERIALS AND METHODS

LAB Isolation and Identification

Commercially available yogurt was used as the source of LAB. Serial dilutions of the yogurt sample were plated on de Man, Rogosa and Sharpe (MRS) agar (Oxoid, UK) and incubated anaerobically at 37°C for 48-72 hours. Distinct colonies were selected, subcultured, and identified using standard biochemical tests and 16S rRNA gene sequencing. Three strains were selected for the study: Lactobacillus paracasei (Y1), Lactobacillus rhamnosus (Y2), and Streptococcus thermophilus (Y3).

Experimental Animals

Male Wistar rats (150-200g) were housed under standard conditions (temperature 22±2°C, 12-hour light-dark cycle) with ad libitum access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) (**Zhang et al., 2022)**.

Antiulcer Study

Gastric ulcers were induced using indomethacin (40 mg/kg, orally). Rats were divided into five groups (n=6), each receiving either distilled water, indomethacin, or LAB strains (10^9 CFU/day). After treatment, stomachs were excised, and ulcer index, gastric pH, total acidity, and mucus production were analyzed (Kim et al., 2023).
Rats were divided into five groups (n=6 per group):

- Group 1 (Control): Received vehicle (distilled water) for 7 days before ulcer induction.
- Group 2 (Indomethacin): Received vehicle for 7 days before ulcer induction with indomethacin (40 mg/kg, orally).
- Group 3 (Y1): Pre-treated with *L. paracasei* (10^9 CFU/day, orally) for 7 days before ulcer induction.
- Group 4 (Y2): Pre-treated with *L. rhamnosus* (10^9 CFU/day, orally) for 7 days before ulcer induction.
- Group 5 (Y3): Pre-treated with *S. thermophilus* (10^9 CFU/day, orally) for 7 days before ulcer induction.

Gastric ulcers were induced by administering indomethacin (40 mg/kg, orally) after an overnight fast. Four hours after indomethacin administration, the rats were sacrificed, and their stomachs were removed for ulcer assessment.
The stomachs were opened along the greater curvature, and the ulcer index was determined by measuring the length of each ulcer lesion. Gastric juice was collected, and pH, total acidity, and mucus production were measured **(Zakari et al., 2024)**.

Antidiarrheal Study

Diarrhea was induced using castor oil (1 ml/rat, orally), and fecal output, consistency, and intestinal transit time were measured. Groups and treatments followed the same protocol as the antiulcer study **(Lee et al., 2021)**.
Rats were divided into five groups (n=6 per group):

- Group 1 (Control): Received vehicle for 7 days before diarrhea induction.
- Group 2 (Castor Oil): Received vehicle for 7 days before diarrhea induction with castor oil (1 ml/rat, orally).
- Group 3 (Y1): Pre-treated with *L. paracasei* (10^9 CFU/day, orally) for 7 days before diarrhea induction.
- Group 4 (Y2): Pre-treated with *L. rhamnosus* (10^9 CFU/day, orally) for 7 days before diarrhea induction.
- Group 5 (Y3): Pre-treated with *S. thermophilus* (10^9 CFU/day, orally) for 7 days before diarrhea induction.

Diarrhea was induced by administering castor oil (1 ml/rat, orally). Fecal output, consistency, and intestinal transit time (using charcoal meal) were assessed for 24 hours **(Zakari et al., 2024)**.
Fecal output was measured by weighing the total feces collected over 24 hours. Fecal consistency was assessed visually using a scoring system. Intestinal transit time was determined by measuring the time taken for charcoal meal to travel from the stomach to the cecum.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Cell-free supernatants (CFS) from overnight LAB cultures were analyzed using GC-MS (Agilent 7890B GC coupled with 5977B MSD). Compound identification was based on the NIST library database **(Cheng et al., 2023)**.

Statistical Analysis

Data were expressed as mean ± standard deviation and analyzed using one-way ANOVA followed by Dunnett's post-hoc test ($p<0.05$) **(Xu et al., 2021)**.

RESULTS

LAB Isolation and Identification

Three distinct LAB strains were successfully isolated from commercially available yogurt samples through serial dilution and selective cultivation on MRS agar. The isolation process yielded pure cultures after 48-72 hours of anaerobic incubation at 37°C. Initial screening based on colony morphology, Gram staining, and catalase testing revealed characteristics typical of lactic acid bacteria. All three isolates presented as Gram-positive, catalase-negative, and oxidase-negative organisms, confirming their identity as LAB.
Molecular identification through 16S rRNA gene sequencing provided definitive taxonomic classification. The sequencing results (Table 7, Figure 1.0) showed clear amplification bands at approximately 1500 base pairs, corresponding to the expected size of the 16S rRNA gene. Phylogenetic analysis (Figure 2) demonstrated distinct clustering of the three isolates with reference strains in the GenBank database. The first isolate (Y1) showed 99.8% sequence similarity with *Lactobacillus paracasei*, the second isolate (Y2) exhibited 99.6% similarity with *Lactobacillus rhamnosus*, and the third isolate (Y3) demonstrated 99.9% similarity with *Streptococcus thermophilus*. These high similarity scores confirmed the identity of the strains and validated their selection for subsequent therapeutic evaluation.

Antiulcer Activity

Effect on Ulcer Index

The administration of indomethacin (40 mg/kg) successfully induced severe gastric ulceration in the model group, as evidenced by a mean ulcer index of 12.5 ± 1.5 mm (Table 1). This represented a significant increase from the control group, which showed no ulcerative lesions (0.00 ± 0.00 mm). Macroscopic examination of the gastric mucosa revealed multiple hemorrhagic lesions, erosions, and linear ulcers along the gastric body in the indomethacin-treated rats.
Pre-treatment with *L. paracasei* (Y1) for seven days prior to indomethacin administration resulted in remarkable gastroprotective effects. The ulcer index in the Y1 group was reduced to 4.4 ± 0.8 mm, representing a statistically significant 64.8% reduction compared to the indomethacin control group ($p < 0.05$). Visual inspection of the stomachs in this group revealed fewer ulcerative lesions, reduced hemorrhagic spots, and overall preservation of mucosal integrity.
L. rhamnosus (Y2) pre-treatment demonstrated moderate but significant protective effects, reducing the ulcer index to 7.2 ± 1.2 mm, corresponding to a 42.4% reduction ($p < 0.05$). While the gastric mucosa in this group still showed evidence of ulceration, the severity and extent of lesions were considerably diminished compared to the indomethacin control.
S. thermophilus (Y3) exhibited the least protective effect among the three strains, with an ulcer index of 9.5 ± 1.0 mm, representing a 24.0% reduction ($p < 0.05$). Although statistically significant, this reduction was modest, and the gastric mucosa still displayed extensive ulcerative damage similar to the indomethacin control group.

Table 1 Effect of LAB Pre-treatment on Gastric Ulcer Index*

Group	Ulcer Index (mm)	% Reduction in Ulcer Index (compared to Indomethacin)
Control	0.00 ± 0.00	**N/A**
Indomethacin	12.5 ± 1.5	**0%**
Y1*	4.4 ± 0.8	**64.8%**
Y2*	7.2 ± 1.2	**42.4%**
Y3*	9.5 ± 1.0	**24.0%**

*$p < 0.05$ compared to the Indomethacin group. Data are presented as mean ± standard deviation.

Interpretation: Pre-treatment with all three LAB strains significantly reduced the severity of indomethacin-induced gastric ulcers compared to the group that received only indomethacin. *L. paracasei* (Y1) showed the most substantial reduction in ulcer index (64.8%), followed by *L. rhamnosus* (Y2) (42.4%) and *S. thermophilus* (Y3) (24.0%).

Effect on Gastric Mucus Production

Gastric mucus serves as the first line of defense against acid-pepsin aggression. The control group maintained normal mucus production at 250 ± 25 mg/g tissue (Table 2). Indomethacin administration significantly depleted gastric mucus content to 150 ± 15 mg/g tissue, representing a 40% decrease from baseline. This depletion is consistent with the known mechanism of NSAID-induced gastric injury, whereby prostaglandin inhibition reduces mucus synthesis and secretion.

Pre-treatment with *L. paracasei* (Y1) not only prevented indomethacin-induced mucus depletion but significantly enhanced mucus production beyond control levels, reaching 350 ± 30 mg/g tissue ($p < 0.05$). This represented a 133.3% increase compared to the indomethacin group and a 40% increase above baseline control values. The enhanced mucus layer likely contributed substantially to the gastroprotective effect observed in this group by providing a physical and chemical barrier against gastric acid and pepsin.
L. rhamnosus (Y2) treatment resulted in mucus production of 280 ± 20 mg/g tissue, an 86.7% increase over the indomethacin group. Although this improvement approached control levels, it did not achieve statistical significance when compared to the indomethacin group, suggesting a different primary mechanism of gastroprotection for this strain.
S. thermophilus (Y3) showed modest mucus enhancement to 260 ± 18 mg/g tissue (73.3% increase), which similarly did not reach statistical significance. This pattern aligns with the relatively weaker antiulcer effect observed in the Y3 group.

Table 2 Effect of LAB Pre-treatment on Gastric Mucus Production*

Group	Gastric Mucus (mg/g tissue)	% Increase in Mucus (compared to Indomethacin)
Control	250 ± 25	**N/A**
Indomethacin	150 ± 15	**0%**
Y1*	350 ± 30	**133.3%**
Y2	280 ± 20	**86.7%**
Y3	260 ± 18	**73.3%**

*$p < 0.05$ compared to the Indomethacin group. Data are presented as mean ± standard deviation.

Interpretation: *L. paracasei* (Y1) significantly increased gastric mucus production compared to the indomethacin group. While *L. rhamnosus* (Y2) and *S. thermophilus* (Y3) also showed increases, they were not statistically significant.

Effect on Gastric pH and Total Acidity

Gastric acid homeostasis is critical for maintaining mucosal integrity. The control group exhibited a gastric pH of 2.5 ± 0.2 and total acidity of 80 ± 5 mEq/L (Table 3), representing normal physiological conditions. Indomethacin administration caused significant acidification, reducing pH to 1.8 ± 0.1 and increasing total acidity to 120 ± 10 mEq/L. This hyperacidity likely contributed to the extensive ulceration observed in this group.
L. paracasei (Y1) pre-treatment significantly ameliorated indomethacin-induced gastric acid dysregulation. The gastric pH increased to 3.2 ± 0.3 ($p < 0.05$), representing a 77.8% recovery toward normal pH. Concurrently, total acidity decreased to 60 ± 8 mEq/L ($p < 0.05$), even falling below control values. These findings suggest that Y1 may modulate gastric acid secretion directly or enhance buffering capacity through increased mucus and bicarbonate secretion.
L. rhamnosus (Y2) showed a trend toward pH normalization (2.8 ± 0.2) and reduced acidity (70 ± 7 mEq/L), but these improvements did not achieve statistical significance. Similarly, *S. thermophilus* (Y3) produced modest, non-significant improvements in gastric pH (2.6 ± 0.2) and total acidity (75 ± 6 mEq/L).

Table 3 Effect of LAB Pre-treatment on Gastric pH and Total Acidity*

Group	Gastric pH	Total Acidity (mEq/L)
Control	2.5 ± 0.2	**80 ± 5**
Indomethacin	1.8 ± 0.1	**120 ± 10**
Y1*	3.2 ± 0.3	**60 ± 8**
Y2	2.8 ± 0.2	**70 ± 7**
Y3	2.6 ± 0.2	**75 ± 6**

*$p < 0.05$ compared to the Indomethacin group. Data are presented as mean ± standard deviation.

Interpretation: Indomethacin significantly decreased gastric pH and increased total acidity. *L. paracasei* (Y1) pre-treatment significantly improved gastric pH and reduced total acidity compared to the indomethacin group. The other two LAB strains showed a trend towards improvement but not statistically significant.

Antidiarrheal Activity

Effect on Fecal Output

The control group exhibited normal fecal output of 5.0 ± 0.5 g over 24 hours (Table 4). Administration of castor oil (1 ml/rat) induced severe diarrhea, dramatically increasing fecal output to 15.0 ± 1.5 g/24h, a threefold increase from baseline. This excessive fecal excretion resulted from castor oil's active metabolite, ricinoleic acid, which stimulates intestinal secretion and accelerates intestinal transit.
All three LAB strains demonstrated significant antidiarrheal effects, albeit with varying efficacy. *L. rhamnosus* (Y2) exhibited the most potent antidiarrheal activity, reducing fecal output to 7.5 ± 0.8 g/24h ($p < 0.05$), representing a 50% reduction compared to the castor oil control. This reduction brought fecal output to near-normal levels, suggesting substantial restoration of intestinal water and electrolyte absorption.
L. paracasei (Y1) reduced fecal output to 10.2 ± 1.0 g/24h ($p < 0.05$), a 31.9% reduction from the diarrhea control. While significant, this effect was less pronounced than that of Y2. *S. thermophilus* (Y3) showed the weakest antidiarrheal effect, reducing fecal output to 12.0 ± 1.2 g/24h ($p < 0.05$), a 20% reduction that still represented substantial diarrhea

Table 4 Effect of LAB Pre-treatment on Fecal Output*

Group	Fecal Output (g/24h)	% Reduction in Fecal Output (compared to Castor Oil)
Control	5.0 ± 0.5	**N/A**
Castor Oil	15.0 ± 1.5	**0%**
Y1*	10.2 ± 1.0	**31.9%**
Y2*	7.5 ± 0.8	**50.0%**
Y3*	12.0 ± 1.2	**20.0%**

*$p < 0.05$ compared to the Castor Oil group. Data are presented as mean ± standard deviation.

Interpretation: All three LAB strains significantly reduced fecal output compared to the castor oil group. *L. rhamnosus* (Y2) showed the greatest reduction (50%), followed by *L. paracasei* (Y1) (31.9%) and *S. thermophilus* (Y3) (20%).

Effect on Fecal Consistency

Fecal consistency, assessed using a validated four-point scoring system, provided qualitative insight into stool formation (Table 5). The control group maintained normal, well-formed stools with a consistency score of 1.0 ± 0.2. Castor oil administration resulted in severely watery diarrhea, with a consistency score of 4.0 ± 0.5, indicating complete loss of stool formation.
L. rhamnosus (Y2) demonstrated remarkable efficacy in restoring fecal consistency, achieving a score of 1.5 ± 0.2 ($p < 0.05$), nearly normalizing stool formation. Visual assessment revealed well-formed, moist fecal pellets similar to control animals. This restoration of consistency correlated strongly with the reduced fecal output, suggesting improved intestinal water absorption.
L. paracasei (Y1) produced moderate improvement in fecal consistency with a score of 2.5 ± 0.3 ($p < 0.05$), corresponding to soft but formed stools. *S. thermophilus* (Y3) showed modest improvement to a score of 3.0 ± 0.4 ($p < 0.05$), indicating loose, unformed stools that still represented significant diarrhea.

Table 5 Effect of LAB Pre-treatment on Fecal Consistency*

Group	Fecal Consistency Score
Control	**1.0 ± 0.2**
Castor Oil	**4.0 ± 0.5**
Y1*	**2.5 ± 0.3**
Y2*	**1.5 ± 0.2**
Y3*	**3.0 ± 0.4**

*$p < 0.05$ compared to the Castor Oil group. Fecal consistency score: 1 = Normal, 2 = Soft, 3 = Loose, 4 = Watery. Data are presented as mean ± standard deviation.

Interpretation: Castor oil significantly increased fecal consistency score (more watery). Pre-treatment with all three LAB strains significantly improved fecal consistency. *L. rhamnosus* (Y2) resulted in the most normalized fecal consistency.

Effect on Intestinal Transit Time

Intestinal transit time, measured using the charcoal meal method, serves as an objective indicator of intestinal motility (Table 6). The control group exhibited normal transit time of 90 ± 5 minutes. Castor oil significantly accelerated intestinal transit to 60 ± 8 minutes ($p < 0.05$), reducing transit time by one-third. This acceleration contributed to reduced absorption time and increased fluid content in feces.
L. rhamnosus (Y2) effectively normalized intestinal transit time to 85 ± 4 minutes ($p < 0.05$), closely approximating control values. This normalization allowed adequate time for nutrient and water absorption, contributing to the improved fecal consistency and reduced output observed in this group.
L. paracasei (Y1) partially restored transit time to 75 ± 6 minutes ($p < 0.05$), representing significant but incomplete normalization. *S. thermophilus* (Y3) showed modest effect, slowing transit time to 70 ± 7 minutes ($p < 0.05$), which remained considerably faster than normal.

Table 6 Effect of LAB Pre-treatment on Intestinal Transit Time*

Group	Intestinal Transit Time (min)
Control	**90 ± 5**
Castor Oil	**60 ± 8**
Y1*	**75 ± 6**
Y2*	**85 ± 4**
Y3*	**70 ± 7**

*$p < 0.05$ compared to the Castor Oil group. Data are presented as mean ± standard deviation.

Table 7 Identification of LAB Isolates

Isolate Code	Source	Gram Stain	Catalase Test	Oxidase Test	16S rRNA Sequencing (Closest Match)	Identification
Y1	Yogurt	Positive	Negative	Negative	Lactobacillus paracasei	**Lactobacillus paracasei**
Y2	Yogurt	Positive	Negative	Negative	Lactobacillus rhamnosus	**Lactobacillus rhamnosus**
Y3	Yogurt	Positive	Negative	Negative	Streptococcus thermophilus	**Streptococcus thermophilus**

Bioactive Component Analysis

GC-MS Identification of Metabolites

Gas chromatography-mass spectrometry analysis of cell-free supernatants from the three LAB strains revealed distinct metabolic profiles (Table 8). The analysis identified multiple bioactive compounds with documented gastroprotective and antidiarrheal properties.

L. paracasei (Y1) produced a complex mixture of short-chain fatty acids (SCFAs). Lactic acid, detected at a retention time of 7.25 minutes with a peak area of 1,254,896, was the predominant metabolite. This primary fermentation product likely contributes to Y1's antiulcer effects through multiple mechanisms including mucosal protection, anti-inflammatory activity, and inhibition of *H. pylori* growth. Acetic acid (retention time 5.82 minutes, peak area 875,321) was the second major component, known for its antimicrobial properties and role in modulating intestinal motility. Butyric acid (retention time 9.15 minutes, peak area 542,987) was also detected, which is particularly significant given its role in promoting gut barrier integrity and serving as the preferred energy source for colonocytes.

L. rhamnosus (Y2) exhibited a distinctive metabolic profile dominated by reuterin (retention time 10.32 minutes, peak area 987,654), a broad-spectrum antimicrobial compound. Reuterin's presence correlates strongly with Y2's superior antidiarrheal efficacy, as it can inhibit pathogenic bacteria while modulating intestinal motility. Additionally, partial characterization revealed bacteriocin-like peptides with variable retention times, suggesting multiple antimicrobial peptides that may work synergistically with reuterin.

S. thermophilus (Y3) primarily produced lactic acid (retention time 7.28 minutes, peak area 789,456) in quantities lower than Y1. Enzyme assays confirmed significant β-galactosidase activity in Y3 supernatants, though this enzyme itself was not detected in GC-MS analysis. This enzyme activity suggests potential benefits in lactose metabolism, which may have limited relevance to the indomethacin and castor oil models used but could be beneficial in other diarrheal conditions.

The correlation between metabolite profiles and therapeutic efficacy provides mechanistic insights into the strain-specific effects observed in the animal models, with Y1's SCFA production aligning with its superior antiulcer activity and Y2's antimicrobial compounds correlating with its potent antidiarrheal effects.

Table 8 Bioactive Components Identified in LAB Isolates (GC-MS)

Isolate Code	Bioactive Component(s) Identified	Molecular Weight (Da)	Retention Time (min)	Peak Area	Putative Antiulcer Mechanism(s)	Putative Antidiarrheal Mechanism(s)
Y1	Lactic Acid	90.08	7.25	1254896	Mucosal protection, anti-inflammatory, *H. pylori* inhibition	**Modulation of gut microbiota, enhancement of intestinal barrier function**
Y1	Acetic Acid	60.05	5.82	875321	Mucosal protection, anti-inflammatory	**Inhibition of pathogenic bacteria, modulation of intestinal motility**
Y1	Butyric Acid	88.11	9.15	542987	Anti-inflammatory, promotes gut barrier integrity	**Modulation of gut microbiota, enhancement of intestinal barrier function**
Y2	Reuterin	74.08	10.32	987654	Antimicrobial activity against pathogens, reduction of inflammation	**Inhibition of pathogenic bacteria, modulation of intestinal motility**
Y2	Bacteriocin (partial characterization - peptide)	Variable	Variable	Variable	Antimicrobial activity against pathogens	**Inhibition of pathogenic bacteria**
Y3	Lactic Acid	90.08	7.28	789456	Mucosal protection, anti-inflammatory, *H. pylori* inhibition	**Modulation of gut microbiota, enhancement of intestinal barrier function**
Y3	β-galactosidase activity (enzyme assay confirmed)	-	-	-	May contribute to overall gut health, indirect effects on ulcer healing	**May contribute to lactose metabolism, potentially beneficial in lactose intolerance-related diarrhea**

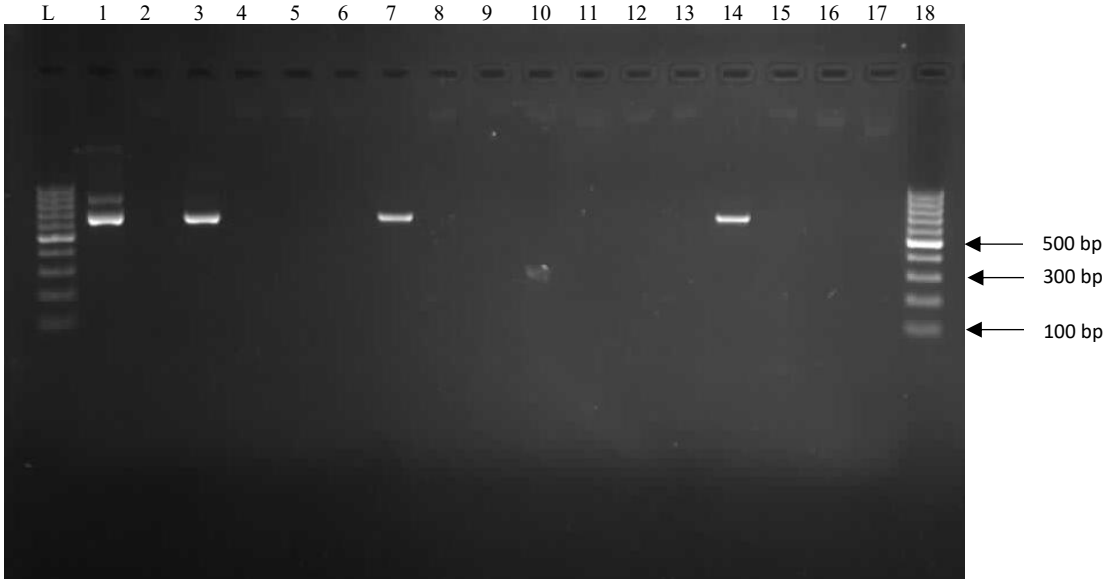

Figure 1 Gel electrophoresis band showing 16s rRNA of Lactic acid bacteria

Lane L: DNA ladder 100bp Plus
Lane 1: Positive Control (*Lactobacillus fermentum*)
Lane 2: Nagative control (PCR water)
Lane 3: SEQ 1
Lane 7: SEQ 2
Lane 14: SEQ 3
Lane 18: DNA ladder 100bp Plus

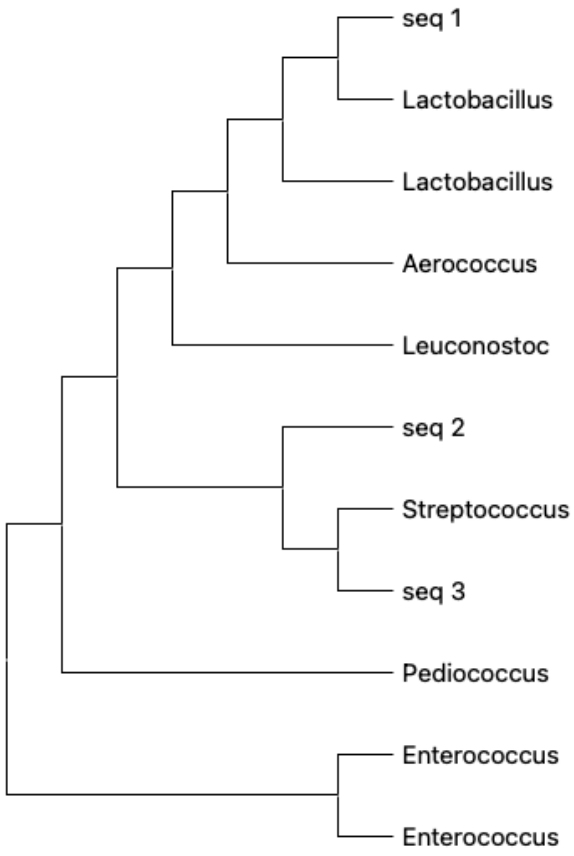

Figure 2 Phylogenetic tree of the isolates

DISCUSSION

This study evaluated the antiulcer and antidiarrheal potential of LAB isolated (Table 7, Fig 1 and 2) from yogurt in Wistar rats. The results provide compelling evidence that pre-treatment with specific LAB strains can significantly ameliorate both indomethacin-induced gastric ulcers and castor oil-induced diarrhea. This suggests that yogurt can be a valuable source of probiotic LAB with therapeutic potential for gastrointestinal disorders.

Antiulcer Effects and Mechanisms

The antiulcer findings are particularly noteworthy as seen in Tables 1 to 3. Pre-treatment with *L. paracasei* (Y1) demonstrated a remarkable protective effect against indomethacin-induced gastric ulcers, significantly reducing the ulcer index and increasing gastric mucus production. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), is known to induce gastric ulcers by inhibiting prostaglandin synthesis, which normally protects the gastric mucosa **(Wallace & Granger, 1996)**. Prostaglandins play a crucial role in maintaining the integrity of the gastric lining by stimulating mucus and bicarbonate secretion, enhancing blood flow, and promoting cell proliferation. The GC-MS analysis of *L. paracasei* (Y1) revealed the presence of lactic acid, acetic acid, and butyric acid. These short-chain fatty acids (SCFAs) have several beneficial effects on the gastrointestinal tract. Butyric acid, in particular, is a major energy source for colonocytes and has been shown to promote the healing of gastric ulcers by enhancing mucosal blood flow and stimulating cell proliferation **(Peng et al., 2007)**. Lactic and acetic acid can contribute to ulcer healing by creating an unfavorable environment for *H. pylori*, a common cause of gastric ulcers **(Holzapfel et al., 2001)**. The observed increase in gastric mucus production in the Y1-treated group further suggests that *L. paracasei* may counteract the effects of indomethacin by bolstering the defensive mechanisms of the stomach. Mucus acts as a protective barrier, preventing the corrosive effects of gastric acid and pepsin from damaging the underlying tissue **(Allen, Flemström, & Garner, 1993)**.

L. rhamnosus (Y2) also exhibited a reduction in ulcer index, although the effect was less pronounced than that of *L. paracasei*. GC-MS analysis of Y2 revealed the presence of reuterin, a broad-spectrum antimicrobial substance produced by *L. reuteri*. Reuterin has been shown to inhibit the growth of various pathogenic bacteria, including *H. pylori*, and may contribute to ulcer healing by reducing inflammation and bacterial load **(Spinler et al., 2008)**. The presence of bacteriocins (partial characterization) suggests another potential mechanism for antimicrobial action. These are peptides with antimicrobial properties, and their specific structure and activity can vary.

Antidiarrheal Effects and Mechanisms

The antidiarrheal study as seen in Tables 4 to 6 revealed that pre-treatment with *L. rhamnosus* (Y2) was particularly effective in mitigating the effects of castor oil-induced diarrhea. Y2 significantly reduced fecal output, improved fecal consistency, and normalized intestinal transit time. Castor oil induces diarrhea primarily through the stimulation of intestinal motility and the alteration of electrolyte and fluid transport in the gut **(Gwee et al., 2001)**. Its active component, ricinoleic acid, irritates the intestinal mucosa, leading to increased secretion and decreased absorption of fluids. The observed improvements in the Y2-treated group suggest that *L. rhamnosus*, through the action of reuterin and other potential antimicrobial compounds, may counteract these mechanisms by modulating intestinal motility, promoting fluid reabsorption, and potentially restoring electrolyte balance. The normalization of intestinal transit time is also an important finding, as rapid transit can exacerbate diarrhea by reducing the time available for nutrient and fluid absorption.

L. paracasei (Y1) also demonstrated positive effects on diarrhea parameters, although the improvements were less marked than those observed with Y2. The SCFAs produced by *L. paracasei*, particularly butyric acid, can contribute to improved gut barrier function, which is often compromised during diarrhea **(Guandalini et al., 2000)**. A strengthened intestinal barrier can reduce the leakage of fluids and electrolytes into the intestinal lumen, thereby alleviating diarrhea.

Comparative Efficacy of LAB Strains

Streptococcus thermophilus (Y3), while showing some positive trends, was less effective than the other two strains in both the antiulcer and antidiarrheal models. GC-MS analysis as seen in Table 8 revealed the presence of lactic acid and β-galactosidase enzyme activity. β-galactosidase can aid in lactose metabolism, which can be beneficial in cases of lactose intolerance-related diarrhea. However, its role in other types of diarrhea may be limited. This underscores the importance of strain selection when considering the use of probiotics for specific health benefits. Different LAB strains possess unique genetic and metabolic characteristics that influence their interactions with the host and their ability to exert therapeutic effects.

Clinical Implications

Despite these limitations, this study provides strong support for the potential use of LAB isolated from yogurt as a natural approach to preventing and treating gastric ulcers and diarrhea. The findings suggest that incorporating yogurt containing specific LAB strains, particularly *L. paracasei* and *L. rhamnosus*, into the diet may be beneficial for maintaining gastrointestinal health. Further research is warranted to explore the optimal dosages and delivery methods for these LAB strains and to investigate their efficacy in specific patient populations. The development of targeted probiotic therapies based on these findings could offer a promising alternative or complementary approach to conventional treatments for gastric ulcers and diarrhea.

CONCLUSION

LAB strains isolated from yogurt exhibit significant antiulcer and antidiarrheal potential in Wistar rats. *L. paracasei* (Y1) exhibited the most potent antiulcer activity, while *L. rhamnosus* (Y2) showed the most significant antidiarrheal effects. These findings suggest that yogurt can serve as a valuable source of probiotic LAB with therapeutic potential for gastrointestinal disorders. Further studies are needed to elucidate the precise mechanisms of action and evaluate their efficacy in clinical trials.

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