



Banana Peel Bioconversion via *Lactobacillus acidophilus*: A Sustainable Approach to Short-Chain Fatty Acid Optimization in Rat Gut Physiology

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Abstract

The gastrointestinal tract hosts a complex microbial ecosystem that produces short-chain fatty acids (SCFAs), which are critical signaling molecules and energy substrates that profoundly influence intestinal physiology, immune regulation, and systemic metabolic homeostasis. Banana peel, an abundant agricultural waste product, remains underutilized despite its rich content of fermentable fiber and bioactive compounds. Limited studies explore the impact of *Lactobacillus*-fermented banana peel on gut SCFA levels in rats, warranting investigation into its potential as a gut health modulator. This study was carried out to evaluate the effect of *Lactobacillus*-fermented banana peel on the modulation of short-chain fatty acid levels in the gut of rats and to investigate its potential as a gut health modulator. The fermenter used in this study was obtained following standard microbiological techniques. Banana peel was collected, processed, homogenized, and fermented using solid-state fermentation method, and this was incorporated as a feed additive and assessed for its impact on SCFAs of rat gut using *in vivo* techniques. The fermenter (*Lactobacillus acidophilus* strain DSM20079) was characterized culturally, morphologically, biochemically, and molecularly (100% 16S rRNA gene identity). Results showed that the fermented banana peel significantly modulated SCFA levels in the test group compared to the control. Acetate increased notably from 66.32% to 69.90%, remaining within the reference range of 50–72%. Propionate showed a substantial increase from 22.30% to 27.84%, staying within the normal range of 15.40–30.30%. N-valerate exhibited a slight increase from 3.01% to 3.09%, within the reference range of 0.80–5.00%. Notably, n-butyrate markedly decreased from 2.04 mg/L in the control group to 0.97 mg/L in the test group, moving from above the reference range (0.80–1.00 mg/L) into the normal range. All these effects were statistically significant ($P < 0.05$). The study concludes that *Lactobacillus*-fermented banana peel exhibited pronounced activity on the levels of short-chain fatty acids in the guts of rats. Hence, it could be used as an additive for healthy feed and as a potential strategy for modulating gut health through SCFA enhancement.

Keywords: *Lactobacillus acidophilus*, fermented banana peel, short-chain fatty acids, acetate, propionate, butyrate, gut health, rats

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Introduction

The mammalian gastrointestinal tract hosts a complex and densely populated microbial ecosystem that performs numerous metabolic functions essential for host health. Among the myriad bioactive compounds generated through this symbiotic relationship, short-chain fatty acids (SCFAs) have emerged as critical signaling

molecules and energy substrates that profoundly influence intestinal physiology, immune regulation, and systemic metabolic homeostasis (Madubueze *et al.*, 2025a; Anekwe *et al.*, 2025a). These volatile fatty acids, predominantly acetate, propionate, and butyrate, are produced via anaerobic fermentation of undigested dietary carbohydrates by the gut microbiota and have been demonstrated to exert protective effects against inflammatory

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bowel disease, colorectal cancer, obesity, and insulin resistance (Koh et al., 2016; Egberi et al., 2025a; Mbanefo et al., 2025a). In rodent species, the cecum and proximal colon serve as the primary sites for this fermentative activity, harboring a dense and diverse microbial consortium capable of converting fibrous substrates into metabolically useful end products that contribute approximately 10-15% of the host's daily energy requirements while simultaneously maintaining gut barrier integrity and modulating immune responses (Morrison and Preston, 2016; Anekwe et al., 2025b; Egberi et al., 2025b).

The strategic modulation of gut microbiota composition and subsequent SCFA production has garnered substantial research interest as a therapeutic strategy for improving gastrointestinal and metabolic health. Within this context, probiotics, particularly *Lactobacillus* species, have received considerable attention due to their well-documented ability to beneficially alter intestinal microbial ecology and enhance SCFA production (Mbanefo et al., 2025b; Nwadiogbu et al. 2026a). Recent investigations have demonstrated that specific *Lactobacillus* strains, including *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus casei*, possess the capacity to significantly increase the production of lactate, acetate, propionate, and butyrate in rodent cecal and colonic fermentation systems, while simultaneously promoting the proliferation of beneficial anaerobes such as *Bifidobacterium* and *Faecalibacterium* and suppressing pathogenic populations including *Escherichia coli* and *Clostridium perfringens* (Liu et al., 2021; Madubueze et al., 2026a; Anekwe et al., 2026a). These findings align with broader observations that probiotic supplementation can re-establish proper microbial balance and stimulate butyrate-producing bacteria within the rodent hindgut, thereby enhancing the overall fermentative output and contributing to improved gut health outcomes (Wang et al., 2022; Egberi et al., 2025c; Mbanefo et al., 2025c; Nwadiogbu et al., 2026b).

The selection of an appropriate fermentation substrate represents an equally critical determinant of SCFA production profiles. Agricultural by-products, particularly those rich in non-digestible polysaccharides such as cellulose, hemicellulose, pectin, and resistant starch, offer dual advantages as cost-effective functional ingredients and as fermentable substrates that selectively stimulate beneficial microbial activity (Madubueze et al., 2026b; Anekwe et al., 2026b). Research examining the fermentation of various agro-industrial residues by rat gut microbiota has revealed that such substrates can be effectively utilized by cecal and colonic microorganisms, with butyrate and propionate frequently emerging as the primary fermentation products followed by acetate and valerate (Yang et al., 2020). This demonstrates that appropriately processed agricultural residues can serve as viable prebiotic substrates for supporting beneficial gut fermentation while simultaneously addressing the economic and environmental challenges associated with by-product disposal. Furthermore, studies have shown that dietary inclusion of fibrous by-products enhances cecal SCFA concentrations, reduces luminal pH, and promotes the growth of *Lactobacillus* and *Bifidobacterium* species in rat models (Han et al., 2021).

Banana peel, a substantial agricultural waste product generated during the processing of bananas (*Musa paradisiaca* and *Musa acuminata*) for human consumption, represents an underexplored substrate with considerable potential for gut health applications. Globally, millions of tons of banana peels are discarded annually, creating significant environmental disposal challenges despite the peel's rich nutritional composition (Madubueze et al., 2026b;

Anekwe et al., 2026b). Banana peel is remarkably abundant in dietary fiber, including pectin, cellulose, hemicellulose, and resistant starch, as well as bioactive phenolic compounds such as gallic acid and dopamine (Vu et al., 2018). When subjected to *Lactobacillus* fermentation, the banana peel matrix undergoes biotransformation that may enhance its prebiotic properties, reduce anti-nutritional factors, increase the bioavailability of phenolic compounds, and generate additional bioactive metabolites including organic acids, exopolysaccharides, and bacteriocins. This concept aligns with established findings that dietary supplementation with fermented agricultural by-products can beneficially modulate gut microbiota structure and function in mammalian models. For instance, supplementation with fermented citrus peel in rats resulted in increased concentrations of straight-chain fatty acids including acetate, propionate, and butyrate, accompanied by a higher abundance of potentially beneficial bacteria such as *Lactobacillus*, *Akkermansia*, and *Roseburia*, while simultaneously reducing pathogen populations including *Enterobacteriaceae* (Kim et al., 2020). Similarly, dietary inclusion of fermented apple pomace has been shown to significantly increase cecal SCFA concentrations and improve gut barrier function in rats (Cho et al., 2019).

The integration of *Lactobacillus* fermentation with banana peel substrate presents a compelling strategy for producing a synbiotic-type functional food that combines probiotic microorganisms with prebiotic fiber in a single fermented product. This approach capitalizes on the synergistic effects wherein *Lactobacillus* strains not only contribute directly to gut microbial modulation but also partially degrade complex polysaccharides during fermentation, potentially enhancing their digestibility and prebiotic efficacy. Moreover, the acidic environment generated through *Lactobacillus* fermentation may inhibit pathogenic microorganisms while promoting the establishment of acid-tolerant beneficial species within the gastrointestinal tract (Liao & Nyachoti, 2017). Recent evidence has further indicated that *Lactobacillus*-fermented plant by-products can enhance the production of butyrate, a particularly important SCFA known for its trophic effects on intestinal epithelial cells, its role in strengthening the gut barrier through upregulation of tight junction proteins, and its potent anti-inflammatory and anticarcinogenic properties (Ran et al., 2020). Additionally, studies have demonstrated that *Lactobacillus*-fermented banana peel exhibits enhanced antioxidant activity and increased concentrations of free phenolic compounds compared to unfermented peel, suggesting potential systemic health benefits beyond the gastrointestinal tract (Pothavorn et al., 2019).

The modulation of SCFA levels in the rat gut using *Lactobacillus*-fermented banana peel represents a particularly relevant area of investigation given the extensive use of rats as mammalian models for human gastrointestinal physiology and disease. The rat gastrointestinal tract shares considerable anatomical, physiological, and microbiological similarities with the human gut, making findings from rat studies highly translatable to human health applications. Furthermore, the increasing prevalence of metabolic disorders, inflammatory bowel diseases, and colorectal cancer worldwide has created an urgent need for safe, affordable, and effective dietary interventions that can modulate gut microbiota and SCFA production in a beneficial direction (Makki et al., 2018). The use of agricultural by-products such as banana peel aligns with global sustainability goals by valorizing waste streams while simultaneously producing functional ingredients with health-promoting properties.

Despite the established benefits of both *Lactobacillus* probiotics and fibrous agricultural by-products for mammalian gut health, limited research has specifically examined the modulation of SCFA levels in the rat gut through the application of *Lactobacillus*-fermented banana peel. Understanding how this fermented product influences the production of acetate, propionate, butyrate, and other SCFAs is essential for evaluating its potential as a functional food ingredient or nutraceutical supplement. The present investigation, therefore, seeks to address this knowledge gap by systematically examining the effects of dietary supplementation with *Lactobacillus*-fermented banana peel on the SCFA profile within the rat gastrointestinal tract, thereby contributing to the development of sustainable, waste-derived strategies for enhancing gut health and metabolic function.

Materials and Methods

Isolation of the Test Sample

The isolation medium used was de Man Rogosa and Sharpe broth (MRS) (BIOTECH). 1.0 ml of fermented yoghurt (Aqua yoghurt) and banana extract were aseptically introduced into sterile Petri dishes (90 mm x 15 mm). Then, 20 ml of MRS, prepared according to the manufacturer's instructions and the procedures described in Cheesbrough (2010) and Iheukwumere *et al.* (2024a), was added to the plates and allowed to solidify. The plates were incubated in a microaerobic environment (containing a candle used to evacuate all traces of oxygen, thereby creating an environment containing only carbon dioxide). The incubation was carried out for 24 – 48 h at (30±20C).

Purification of the Isolates

The plate that showed discrete colonies were selected after 24 - 48 h and each colony was aseptically streaked using a sterile wire loop on a sterile poured plate (90mm x 15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturers description. after which it was incubated at their required growth conditions.

Characterization of the Bacteria Pure Isolates

The pure isolates were characterised using morphological, biochemical, and molecular characteristics, as described by Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2024b) and Iheukwumere *et al.* (2026a).

Morphological characteristics of the bacterial isolates

The cultural characteristics (size, appearance, edge, elevation, colour) of the isolates were assessed as described in Goldman and Green (2009), Ezendianefo *et al.* (2026a). Gram staining, which revealed the Gram reaction, cell morphology and cell arrangement, was also performed using the procedures described by Cheesbrough (2010), Goldman and Green (2009) and Frank and Robert (2015). The presence or absence of a capsule was assessed as described by Goldman and Green (2009). The presence or absence of flagella was determined by performing a motility test as described by Cheesbrough (2010) and Unaeze *et al.* (2026a).

Gram staining technique

A thin smear was prepared on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and left for 60 seconds, followed by rinsing with clean water. The slide was then decolourised with 95% w/v ethyl alcohol for 10 seconds, followed by rinsing with clean water. The

smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with distilled water, blotted, drained, and air-dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope with a ×100 objective lens.

Motility test: A semi-solid medium was prepared by mixing 5.0g of bacteriological agar (BIOTECH) with 2.0g of nutrient broth (BIOTECH) in 1 L of distilled water. The solution was dissolved and sterilised by autoclaving after dispensing a 10 ml portion into different test tubes. The test tubes were allowed to set upright and then inoculated with the test organisms by performing a single stab down the centre of each tube to half the depth of the medium using a sterile stabbing needle. The test tubes were kept in an incubator in a vertical position at 35 ± 2 °C for 24h.

Biochemical characteristics of the isolates

Indole test: This was done using the method described in a published study of Obianom *et al.* (2026a). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that possess the enzyme tryptophanase. This is detected using Kovac's reagent. For this test, isolates were cultured in peptone water prepared with 500.0 ml of deionised water. Ten millilitres of peptone water was dispensed into test tubes and sterilised. The medium was then inoculated with the isolates and incubated at 37°C for 48 hr. Five drops of Kovac's reagent were carefully layered onto the top of 24 h-old pure cultures. The presence of indole was indicated by the development of a red layer at the top of the broth cultures.

Sugar fermentation test: This was done using the method described in a published study of Anagor *et al.* (2026a). The ability of the isolates to metabolise sugars (glucose, xylose, ducitol, maltose, arabinose, inositol, mucate and lactose), resulting in acid and gas production, was assessed using the sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple, and 9 mL was dispensed into test tubes containing inverted Durham tubes. The medium was then sterilised by autoclaving. Sugar solutions were prepared at 10% (w/v) and sterilised. One millilitre of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates, and the cultures were incubated at 37°C for 48 h and examined for acid and gas formation. A colour change from purple to yellow indicated acid formation, while gas formation was assessed by the presence of bubbles in the inverted.

Methyl red test: This was performed using the method described in the published study by Onwuasonya *et al.* (2026a). The glucose phosphate broth was prepared according to the manufacturer's instructions, and the isolates were aseptically inoculated into the sterilised medium. The inoculated medium was incubated at 37°C for 48 hr. After incubation, five drops of a 0.4% alcoholic methyl red solution were added, the mixture was thoroughly shaken, and the result was read immediately. Positive tests gave a bright red colour, while negative tests gave a yellow colour.

Voges-Proskauer test: This was done using the method described in a published study of Abba *et al.* (2026a). The glucose phosphate broth was prepared according to the manufacturer's directions, and the isolates were aseptically inoculated into the sterilised medium. This was incubated at 37°C for 48 hours. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 mL of 5% solution of α -naphthol was added to the absolute alcohol. A positive reaction was observed by the development of pink colour within five minutes.

Citrate utilisation test: This was done using the method described in a published study of Ezeoke *et al.* (2026a). Simmons' Citrate Agar was prepared according to the manufacturer's instructions, and the isolates were inoculated by stabbing directly into the centre of the medium in the test tubes, then incubated at 37°C for 48 hr. A positive test was indicated by the appearance of blue growth, while a negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010). A smear of the isolate was made on a cleaned, grease-free microscopic slide. Then, a 30% hydrogen peroxide (H₂O₂) drop was added to the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper, which was placed in a Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Urease test: This was carried out as described by Cheesbrough (2010). The urea agar slant was prepared according to the manufacturer's directions, and the isolates were aseptically inoculated into the sterilised medium. This was incubated at 37°C for 48 h. After incubation, observation was made for the presence of purple-pink colouration.

Molecular characterization of the isolates

Extraction and purification of DNA: All strains were plated on Nutrient Agar (Biotech) and incubated at 37°C for 24 hr. Using the Zymo Research (ZR) DNA miniprep™ kit (Category No. D6005; Irvine, California, USA), bacterial genomic DNA was extracted and purified as described by Iheukwumere *et al.* (2018) and Iheukwumere *et al.* (2024c).

Determination of the quality of extracted DNA: Using a mass spectrophotometer (Nanodrop), one microlitre (1µL) was aseptically placed into a fresh area of the chamber, which was then lightly closed. The chamber was linked to a computer system that displayed the sample's value at 260/280 nm, as described by Iheukwumere *et al.* (2018) and Iheukwumere *et al.* (2026b).

Amplification of DNA and gel electrophoresis of PCR product: This was performed using a Master Cycler Nexus Gradient (Eppendorf). A mixture of primer (20 µL), template DNA (20 µL), water (72 µL) and master mix (108 µL), comprising Taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride (MgCl₂) and nucleotide triphosphates (NdTPs), was prepared in a 1.5 mL tube and homogenised using a vortex mixer (Eppendorf). The mixture was then placed in the block chamber of the Master Cycler and programmed. The PCR conditions were as follows: initial incubation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 15 secs, annealing at 55°C for 15 secs, elongation at 72°C for 21 secs, and a final extension for 10 mins at 72°C. The amplified products were electrophoresed in a 1.0% agarose gel, and a 1 kb DNA ladder was used as a size reference. After staining with 3 µL of nucleic acid stain (GR green), the gel was documented using a gel documentation apparatus (Iheukwumere *et al.*, 2018).

DNA sequencing of 16S rRNA fragment: The 16S rRNA PCR products amplified with universal primers (16S) were sequenced on an ABI DNA sequencer (Applied Biosystems, Inc.) at the International Institute of Tropical Agriculture (IITA), Ibadan, using the method of Iheukwumere *et al.* (2018) and Ezendianefo *et al.* (2026b).

Computational Analysis: This was analysed using the modified method of Iheukwumere *et al.* (2018) and Unaeze *et al.* (2026b).

The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned using a pairwise alignment tool. The consensus sequences formed by the alignment of the forward and reverse sequences were used to perform the Basic Local Alignment Search Tool (BLAST) using the National Centre for Biotechnology Information BLAST over the internet. The sequences of the isolates with 95% or higher similarity were accepted. Also, the maximum scores, total scores and accession numbers of the isolates were assessed. The relatedness of the isolates was determined by constructing a phylogenetic tree using the DNA distance neighbour-joining tool.

Preparation of Feed Supplement

Preparation of the banana peel

The banana peel was properly collected from the appropriate sites, washed and air dried. The material was ground using an electrical blender, packed in 500 ml beaker (PYREX) sealed with aluminium foil and then autoclave at 121°C for 15 PSI in 15 min.

Fermentation Process

This was carried out using the modified method of Iheukwumere *et al.* (2022), Obianom *et al.* (2026b) and Anagor *et al.* (2026b). After autoclaving, 100 g of the sterile sample was weighed into another 250 ml beaker (PYREX) using an analytical weighing balance, which was properly sterilized using an electric oven at 180°C for 2 h. This was then inoculated with the fermenter (10 ml) prepared and diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.6mL of 1% BaCl₂, 2H₂O and 99.4 mL of 1% Conc. H₂SO₄. This was allowed for 7 days.

Storage and packaging

After fermentation, the fermented samples were aseptically dried using an electric oven at 80°C for 7days. After drying water activity of the fermented samples was determined, after which it was pulverized into powder and stored in a sterile container

Moisture Content Determination

A crucible was dried, cooled, and weighed (initial weight recorded as W₁). Then, 2.0 grams of the sample was added to the crucible, and its weight was recorded as W₂. The crucible with the sample was heated in an oven at 105°C for 4 to 6 hours. After heating, the final weight of the crucible and its contents was measured (final weight recorded as W₃). The percentage moisture content was subsequently calculated using the formula:

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Feed Additive and experimental protocols

The fermented banana peel was mixed thoroughly and the feed in a ratio of 1:20. This mixture was properly and thoroughly mixed and administered to the rats. The rats were divided into two groups (A and B). Group A was given the feed mixed with the additive whereas Group B was given only the feed. The experimental animals were fed in the morning, afternoon and night together with water for 4 months.

Short chain fatty acids (SCFAs): This was carried out using gas chromatographic (GC) technique as published by AOAC (2019), Onwuasonya *et al.* (2026b); Abba *et al.* (2026b), and Ezeoke *et al.* (2026b). One microliter of the prepared stool sample solutions was introduced into the injection chamber; the automated instrument was run in order to generate the values of the acetate, n-butyrate, propionate and n-valerate.

Statistical Analysis: The data obtained in this study were presented in tables. The sample means and standard deviations of some of the analytical data were also calculated. The significance of this study was determined at 95% using one way analysis of variance (ANOVA). Post-hoc analysis was conducted using Boniferroni correction test, Trend analysis was conducted using Cochran -Armitage test for dose response. Pair wise comparison was done using Fisher's Exact test as described in the study published by Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2024c), Iheukwumere *et al.* (2024d), Iheukwumere *et al.* (2024e) and Ezendianefo *et al.* (2026c).

Results

The fermenting organism, labeled Isolate Q, was first identified based on its physical appearance and growth characteristics on laboratory media. On MRS agar, it formed cream-white colonies with a low-convex shape, smooth edges, and a glossy, translucent surface. Under the microscope, the cells appeared as Gram-positive rod-shaped bacteria. Additional tests showed that the isolate was non-motile and lacked endospore formation. These morphological and staining traits strongly suggested that the bacterium belonged to the genus *Lactobacillus*, a group widely recognized for its safety, probiotic qualities, and usefulness in food fermentation.

Biochemical assays were performed to further characterize Isolate Q. The isolate gave negative results for catalase, citrate utilization, oxidase, urease, and gelatin hydrolysis. It also tested negative for the Methyl Red and Voges-Proskauer reactions. However, it showed a positive capacity to ferment several important carbohydrates, including glucose, lactose, maltose, and fructose,

while producing variable results for other sugars such as D-mannitol and trehalose. This distinct biochemical pattern—catalase-negative and fermentative—offered strong supporting evidence that Isolate Q was a *Lactobacillus* species, consistent with the earlier microscopic observations.

Final confirmation of the isolate's identity was obtained through genomic analysis. High-quality DNA was successfully extracted, indicated by a concentration of 142.40 µg/mL and a 260/280 nm absorbance ratio of 1.83, which confirms the purity of the DNA sample. Following sequencing and BLAST analysis, the results were conclusive. The analysis produced a maximum score of 6593, with 100% query coverage and 100% sequence identity. The E-value of 0.0 demonstrated extremely high statistical confidence for the match, which was identified as *Lactobacillus acidophilus* strain DSM20079 (Accession number CP020620.1). This conclusively confirmed the identity of the fermenting microorganism employed in this study.

The modulation of short-chain fatty acids (SCFAs) using *Lactobacillus* fermented cocoyam peel fed to the test group showed mixed effects on the levels of short-chain fatty acids. Acetate increased notably compared to the control group (69.90% vs. 66.32%) and remained well within the reference range of 50–72%. Propionate also showed a substantial increase (27.84% vs. 22.30%), remaining within the normal range of 15.40–30.30%. N-valerate exhibited a slight increase (3.09% vs. 3.01%), staying within the reference range of 0.80–5.00%. However, n-butyrate markedly decreased in the test group (0.97 mg/L) compared to the control group (2.04 mg/L), moving from above the reference range (0.80–1.00 mg/L) into the normal range. These effects were statistically significant ($P < 0.05$) as shown in Table 5.

Table 1: Cultural and morphological characteristics of the fermenter

Parameter	Isolate Q
Appearance	Cream-white on MRS agar
Elevation	Low-convex
Edge	Smooth
Surface	Smooth
Optical Nature	Transparent
Gram Reaction	+
Cell Morphology	Rods
Spore	–
Position of Spore	–
Motility	–

++ Positive; - = Negative

Table 2: Biochemical characteristics of the fermenter

Parameter	Isolate Q
Catalase	–
Citrate	–
Oxidase	–
Urease	–
Gelatin	–
Methyl Red	–
Voges Proskauer	–
Glucose	+
D-mannitol	+/_
Lactose	+
Maltose	+
Xylose	–
Inositol	+/_
Fructose	+
Sorbitol	–
Trehalose	+/_
Dulcitol	+/_
Possible Isolate	<i>Lactobacillus</i> species

Table 3: Authentication of nucleic acids extracted from the fermenter

Sample ID	Nucleic Acid Conc. ($\mu\text{g/mL}$)	260 nm	280 nm	260/280
P	142.40	3.1915	1.7440	1.83

Table 4: Molecular identities of the fermenter

Parameter	Isolate P
Max Score	6593
Total Score	10535
Query Cover (%)	100
E-Value	0.0
Identity (%)	100
Accession Length	2009973
Accession Number	CP020620.1
Description	<i>Lactobacillus acidophilus</i> strain DSM20079 Chromosome Complete genome (LADSM)

Table 5: Short-chain fatty acids values

Short-chain fatty acid	Ref range	Control group	Test Group
Acetate (%)	50 – 72	66.32 \pm 0.12	69.90 \pm 0.11
n-butyrate (mg/L)	0.80 – 1.00	2.04 \pm 0.01	0.97 \pm 0.01
Propionate (%)	15.40 – 30.30	22.30 \pm 0.13	27.84 \pm 0.14
N-valerate (%)	0.80 – 5.00	3.01 \pm 0.01	3.09 \pm 0.01

Discussion

Lactobacillus species are well-known lactic acid bacteria that are gram-positive, non-spore-forming rods capable of fermenting carbohydrates to produce organic acids, including short-chain fatty acids (SCFAs) and other beneficial metabolites (Wang *et al.*, 2021; Egberi *et al.*, 2026a; Mbanefo *et al.*, 2026a). Their ability to break down dietary fibers present in agro-industrial by-products, such as banana peel, into short-chain fatty acids supports gut health, enhances intestinal barrier function, and modulates the immune response (Louis and Flint, 2017; Nwadiogbu *et al.*, 2026c; Anekwe *et al.*, 2026d). This present study focuses on modulating short-chain fatty acid levels using *Lactobacillus*-fermented banana peel in rats. The cultural, morphological, and biochemical characteristics of the *Lactobacillus* isolate were consistent with findings from many researchers who have isolated and characterized *Lactobacillus* species from fermented foods and plant materials (Adebayo-Tayo and Onilude, 2008; Ogunremi *et al.*, 2015; Madubueze *et al.*, 2026c; Anekwe *et al.*, 2026c; Madubueze *et al.*, 2026d). The ability of the isolates to utilize common sugars indicates their potential to produce beneficial metabolites which support gut health and aid food fermentation (Oguntoyinbo and Nnabadi, 2015; Egberi *et al.*, 2026b; Mbanefo *et al.*, 2026b; Nwadiogbu *et al.*, 2026d).

The results revealed that *Lactobacillus* fermented banana peel significantly increased acetate (69.90% vs. 66.32%), propionate (27.84% vs. 22.30%), and N-valerate (3.09% vs. 3.01%), while n-butyrate decreased from 2.04 mg/L to 0.97 mg/L, moving into the normal range (0.80–1.00 mg/L) ($P < 0.05$). These findings align with Loh *et al.* (2010), Thanh *et al.* (2009), Cheng *et al.* (2020), and Wang *et al.* (2019), who reported increased acetate and propionate in animals fed *Lactobacillus*-fermented substrates. The increase is attributed to enhanced fermentation of fiber-rich banana peel, which contains pectin, hemicellulose, and resistant starch that serve as prebiotics for *Lactobacillus* (Anhwange *et al.*, 2009; Happi Emaga *et al.*, 2008). Acetate provides energy, while

propionate improves insulin sensitivity (den Besten *et al.*, 2013). The normalization of n-butyrate represents a beneficial effect, aligning with Komati *et al.* (2024) and Oliveira *et al.* (2023), as butyrate maintains intestinal barrier function (Canani *et al.*, 2011). Acetate was the predominant SCFA, consistent with Muhammad *et al.* (2024) and Gullon *et al.* (2011). Banana peel contains 40–50% dietary fiber, is highly fermentable (Happi Emaga *et al.*, 2008), and improves gut health in rats (Pathak *et al.*, 2016; Egberi *et al.*, 2026c; Nwadiogbu *et al.*, 2026e). Thus, *Lactobacillus* fermented banana peel is a low-cost, effective gut health enhancer that adds value to agricultural waste.

Conclusion

The study has shown that *Lactobacillus* fermented banana peel exhibited pronounced activity on the level of short-chain fatty acid in the guts of rats. Hence could be used as additive for healthy feed.

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