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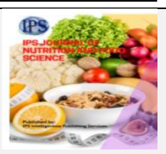

# Corollary of *Lactobacillus* Fermented Plantain Peel on Growth Performance of Rats

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**Abstract**

Plantain peel, a significant agro-waste, represents an untapped resource for animal nutrition, necessitating exploration of its bioconversion into a valuable feed additive. This study investigated the corollary of supplementing rat feed with plantain peel fermented by a novel isolate on growth performance and physiological safety. The fermenting organism was isolated and rigorously characterized as *Lactobacillus acidophilus* (strain DSM20079, Accession CP020620.1) through cultural, biochemical, and molecular (100% identity) analyses. Over a six-week trial, rats fed the fermented supplement exhibited a markedly superior growth trajectory. The final body weight of the test group (324.60 ± 1.51 g) was significantly higher than that of the control group (248.00 ± 1.47 g) ($p < 0.05$). Body Mass Index (BMI) was also higher in the test group (0.66 g/cm²) but remained below the threshold for obesity (0.68 g/cm²). Critically, organ weights (liver, kidney, lungs, heart) and key serum biomarkers for liver (ALT, AST) and kidney (creatinine, urea) function showed no significant differences ($p > 0.05$), confirming the supplement's non-toxic nature. However, assessment of oxidative stress via malondialdehyde (MDA) levels indicated a statistically significant overall increase in the test group ($p = 0.03$), suggesting an elevated oxidative state. In conclusion, *Lactobacillus acidophilus*-fermented plantain peel is a safe and effective probiotic supplement that significantly enhances growth performance in rats without inducing organ toxicity or obesity, though its pro-oxidant effect requires further study to optimize its application as a sustainable nutraceutical.

**Keywords:** *Lactobacillus,* Plantain peel, Fermented, Body-Mass-Index, Probiotic.

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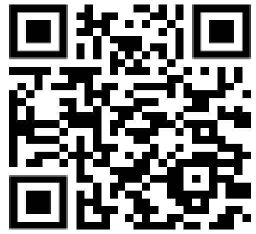

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## Introduction

The global livestock sector stands at a critical juncture, grappling with the formidable challenge of meeting escalating demand for animal protein amidst profound resource constraints and environmental imperatives. With the global population projected to exceed 9 billion by 2050, the pressure to intensify animal production systems is immense, yet this intensification is inextricably linked to the availability and cost of conventional feed resources like maize, soybean, and fishmeal. These ingredients are subject to volatile global markets, geopolitical disruptions, and have significant environmental footprints related to land-use change, water consumption, and greenhouse gas emissions (Henchion *et al.*, 2021; Iheukwumere *et al.*, 2025a; Dim *et al.*, 2025a). Concurrently, the sector faces stringent demands to reduce its ecological impact and operate within planetary boundaries. This confluence of factors has catalysed a paradigm shift towards the development of circular bio-economies within agriculture. The core principle involves transforming linear "take-make-dispose" models into circular systems where waste streams are recaptured as valuable inputs. A primary research frontier, therefore, is the identification and nutritional enhancement of abundant, low-cost, and underutilized agricultural by-products. The successful

integration of these materials into animal diets promises not only to enhance economic resilience by buffering against feed price volatility but also to mitigate environmental pollution, thereby contributing to the sustainability and long-term viability of animal production, particularly in resource-limited regions where such by-products are generated in abundance.

Among the myriad of agro-processing wastes, plantain (*Musa paradisiaca*) peel represents a significant yet largely untapped resource, especially in tropical and subtropical regions where plantain is a staple crop. The processing of plantains for food generates enormous quantities of peel, estimated to constitute about 30-40% of the fruit's total weight. This lignocellulosic biomass is typically discarded through burning, landfilling, or used as low-value compost, practices that contribute to environmental degradation through methane emissions, leachate production, and inefficient resource use (Adeolu and Enesi, 2022; Iheukwumere *et al*., 2022a; Nwike *et al*., 2017). This disposal represents a critical economic and ecological inefficiency. However, a nutritional appraisal reveals that plantain peel is far from inert waste; it contains residual carbohydrates, appreciable amounts of crude fiber, minerals (notably potassium and magnesium), and bioactive compounds such as polyphenols and carotenoids. In its raw, unprocessed state, its direct incorporation into monogastric animal feeds is severely constrained by its high lignin and hemicellulose content, low protein digestibility, and potential presence of anti-nutritional factors, which collectively impair palatability and nutrient bioavailability. This juxtaposition of mass availability and nutritional recalcitrance defines the core problem: transforming a problematic waste stream into a palatable, digestible, and nutritive feed ingredient suitable for non-ruminants like rats and, by extension, poultry and swine (Ekechukwu *et al.,* 2025a; Obianom *et al*., 2024; Dim *et al*., 2025b).

The key to unlocking the latent nutritional value trapped within the fibrous matrix of plantain peel lies in biological pre-treatment, specifically solid-state fermentation (SSF) using lactic acid bacteria, most notably *Lactobacillus* species. This bioprocess leverages the metabolic activity of selected microbial strains to act as natural bioreactors. During SSF, *Lactobacillus* inoculants secrete a suite of exogenous enzymes, including cellulases, xylanases, and pectinases, which systematically degrade complex structural carbohydrates into simpler, more fermentable sugars. This enzymatic breakdown effectively reduces crude fiber content and increases the substrate's metabolizable energy value. Concurrently, fermentation can significantly reduce anti-nutritional factors and enrich the substrate with microbial biomass (single-cell protein), B-vitamins, and beneficial organic acids like lactic and acetic acid (Oladokun *et al*., 2021; Iheukwumere *et al*., 2025b; Dim *et al*., 2025c). These acids lower the substrate's pH, acting as natural preservatives and potentially as gut health modulators when consumed. The process, therefore, does not merely improve digestibility but fundamentally re-engineers the nutritional and functional properties of the peel. The resulting fermented plantain peel (FPP) is hypothesized to be a nutrient-dense, probiotic, and prebiotic feed additive that can enhance diet utilization, support gut health, and contribute to overall animal performance, embodying a classic waste-to-wealth strategy.

In the evaluation of any novel feed ingredient, growth performance remains the paramount and most direct indicator of practical efficacy and economic viability. For growing animals, especially in controlled experimental models like the Albino Wistar rat a well-established proxy for nutritional studies key parameters include body weight gain, feed intake, and feed conversion ratio (FCR). Superior growth performance, evidenced by higher weight gain and a lower (more efficient) FCR, indicates that the animal is effectively utilizing the diet's nutrients for anabolic processes. Improvements in these metrics can be driven by several factors unlocked by fermentation: enhanced nutrient bioavailability, improved palatability leading to increased voluntary feed intake, and the provision of growth-promoting factors like vitamins or bioactive peptides. Furthermore, a healthier gut environment, fostered by the probiotic and prebiotic effects of fermented feed, can reduce maintenance energy costs associated with subclinical inflammation or pathogen challenge, thereby directing more energy towards growth. Assessing these parameters in rats provides critical preclinical data on the ingredient's functionality before potential application in commercial livestock. Such studies are essential to validate the hypothesis that bioprocessing can translate a waste material's chemical potential into tangible biological benefits.

Despite the documented promise of fermentation technology and the established nutritional profile of plantain peel, a significant research gap exists. There is a paucity of controlled, scientific studies specifically investigating the *in vivo* effects of *Lactobacillus*-fermented plantain peel on the growth performance of a standard mammalian model. While some research has explored fermented cassava peel or palm kernel cake in poultry, the unique composition and fermentation dynamics of plantain peel warrant dedicated investigation. Hence, this study is designed to evaluate the corollary of *Lactobacillus* fermented plantain peel on the growth performance of rats.

## Materials and Methods

### Isolation of the Test Sample

The media used for this isolation was de Man Rogosa and Sharpe broth (MRS) (BIOTECH). A 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 which was prepared according to the manufacturers instruction and the procedures described in Cheesbrough (2010), Ekechukwu *et al*. (2025b), Ekesiobi *et al*., (2025), Ezedianafo *et al*., (2025a) was added into the plates, allowed to solidified. The plates were incubated in a microaerophic environment (containing candle used to evacuate all traces of oxygen thereby creating an environment having only carbon iv oxide). The incubation was done for 24 – 48 h at (30±2$^0$C).

### 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 as described by Iheukwumere *et al*. (2020a), Ezedianafo *et al*. (2025b); Idigo *et al*. (2025a), Iheukwumere *et al*. (2025c).

**Characterization of the Bacteria Pure Isolates**

The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* (2017a); Iheukwumere *et al.* (2018a), Ike *et al*. (2025a), Iheukwumere *et al*. (2025d).

**Morphological characteristics of the Bacteria isolates**

The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates were carried out as described in Goldman and Green (2009); Iheukwumere *et al.* (2017b), Iheukwumere *et al*. (2018b), Iheukwumere *et al*. (2020b). The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Cheesbrough (2010), Goldman and Green (2009) Frank and Robert (2015), Iheukwumere *et al*. (2022b), Iheukwumere *et al*. (2023a). The presence or absence of capsule was also carried out as described by Goldman and Green (2009), Ike *et al*. (2025b), Obiefuna *et al*. (2025a). The presence or absence of flagellum was determined by carrying out motility test as described by Cheesbrough (2010), Iheukwumere *et al*., (2017c), Iheukwumere *et al*. (2018c), Iheukwumere and Iheukwumere (2022a).

**Gram staining technique**

A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95% w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Iheukwumere *et al*. (2017d); Iheukwumere *et al*. (2020c), Chude *et al*. (2020), Iheukwumere and Iheukwumere (2022b), Iheukwumere *et al*. (2022c).

**Motility test:** A semi-solid medium prepared by mixing 5.0g of bacteriological agar (BIOTECH) with 2.0g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10 ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at $35\pm2^0$C for 24h (Iheukwumere *et al*., 2017e; Iheukwumere and Iheukwumere, 2022c; Iheukwumere *et al*., 2022d; Idigo *et al*., 2025b).

**Biochemical characteristics of the isolates**

**Indole test:** Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 ml of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator 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 revealed by the development of red layer colouration on the top of the broth cultures as described by Iheukwumere *et al*. (2022e), Iheukwumere and Iheukwumere (2022d), Iheukwumere *et al*. (2023b), Egbe *et al*. (2025a), Ike *et al*. (2025c).

**Sugar fermentation test:** The capability of the isolates to metabolize some sugars (glucose, xylose, ducitol, maltose, arabinose, inositol, mucate and lactose) with the resulting formation of acid and gas or either were carried out using 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 in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution were prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted (Iheukwumere *et al*., 2022f; Iheukwumere and Iheukwumere, 2022e; Egbe *et al.,* 2025b; Idigo *et al*., 2025c).

**Methyl red test:** The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 hr. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution was added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour (Ezedianafo *et al*., 2025c; Ike *et al*., 2025c).

**Voges-Proskauer test:** The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48hr. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of *α*-naphthol was add ed in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes (Egbe *et al*., 2025b; Ekechukwu *et al*., 2025c).

**Citrate utilization test:** The Simmon's Citrate Agar was prepare according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 hr. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original

green colour was retained (Idigo *et al*., 2025d; Ezedianafo *et al*. 2025d).

**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 drop of 30% hydrogen peroxide ($H_2O_2$) was added on the smear. Prompt effervescence indicated catalase production (Idigo *et al*., 2025e; Idigo *et al*., 2025f).

**Oxidase test:** The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in 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), Idigo *et al*. (2025g) and Idigo *et al*. (2025h). The urea agar slant was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into sterilized medium. This was incubated at $37^0$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. By means of the procedures of Zymo Research (ZR) DNA miniprepTM kit, bacterial genomic DNA was then extracted and purified (Category No. D6005; Irvine, California, USA) as described by Iheukwumere *et al.* (2018) Iheukwumere *et al*. (2025e; Idigo *et al*., 2025h).

**Determination of the quality of extracted DNA:** Using mass spectrophotometer (Nanodrop), One micro litre (1µL) was aseptically dropped into a fresh space in the chamber and the chamber was lightly closed which was then linked to a computer system which showed the window that discovered the value of the sample at 260/280nm as described by (Iheukwumere *et al.,* 2018; Iheukwumere *et al*., 2025f; Idigo *et al*., 2025i).

**Amplification of DNA and gel electrophoresis of PCR product:** This was analysed using Master cycler Nexus Gradient (Eppendorf). A mixture of primer (20 µL), template DNA (20µL), water (72 µL) and master mix (108 µL), which comprises taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride ($MgCl_2$) and nucleotides triphosphates (NdTPs), was made in 1.5 mL tube and homogenized using vortex mixer (Eppendorf). This was then positioned in the block chamber of the master cycler and then programmed. The PCR program for 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 final extension period for 10 mins at 72°C. The amplified products were electrophorezed in 1.0% agarose gel and a1kb DNA ladder was used as a size reference. After staining with 3µL of nucleic acid stain (GR green), the gel was documented with gel documentation apparatus (Iheukwumere *et al.,* 2018; Iheukwumere *et al*., 2025g; Idigo *et al*., 2025j; Idigo *et al*., 2025k).

**DNA sequencing of 16s rRNA fragment:** The 16S rRNA amplified PCR products generated from universal primer (16S), was used for the sequencing using ABI DNA sequencer (Applied Biosystem Inc) at International Institute of Tropical Agriculture (IITA), Ibadan using the method of Iheukwumere *et al.* (2018), Iheukwumere *et al*., (2025h), and Idigo *et al*. (2025l), Idigo *et al*., (2025m).

**Computational Analysis:** This was analysed making use of the modified method of Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2025i), Idigo *et al.* (2025n), Iheukwumere *et al*., (2025j). The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned using pair wise 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 National Centre for Biotechnology Information BLAST over the internet. The sequences of the isolates with 95% and above similarities were accepted. Also the maximum scores, total scores and accession numbers of the isolates were assessed. The relatedness of the isolates was determined by tracing their phylogenetic tree using DNA distance neighbour phylogenetic tree tool.

**Preparation of Feed Supplement**

**Preparation of the plantain peel**

The plantain 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 $121^0$C for 15 PSI in 15 min.

**Fermentation Process**

This was carried out using the modified method of Iheukwumere *et al* (2022), Iheukwumere *et al.* (2025k), Iheukwumere *et al.* (2025l). After autoclaving, a 100 g of the sterile sample was weighed into another 250 ml beaker (PYREX) using analytical weighing balance, which was properly sterilized using electric oven at $180^0$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% $BaCl_2$. $2H_2O$ and 99.4 mL of 1% Conc. $H_2SO_4$. This was allowed for 7 days.

**Storage and packaging**

After fermentation, the fermented samples were aseptically dried using an electric oven at $80^0$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:

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

**Experimented Chicks:** A total of twenty four (24) broiler chicks (3 weeks old) were purchased from poultry market located at Ihiala market, Ihiala L. G. A. in Anambra State were used for the study. The chicks were kept in separate, thoroughly cleaned and disinfected house and provided with feeds and water ad libitum. All the chicks were vaccinated against Newcastle disease using Lasota vaccine strains at 6 and 19 days of age, against infectious bronchitis using live H120 strain at 6 days old and also against avian influenza (A1) disease using inactivated H5N1virus vaccine strain at 7 days old. All the vaccines were given via eye drop instillation except (A1) vaccine, which was given through the subcutaneous route at the back of the neck from the folder report collected from the poultry farmer.

**Feed Additive**

The fermented groundnut chaff was mixed with fish meal and the feed in a ratio of 1:20. This mixture was properly and thoroughly mixed and administered to the chicks. The chicks 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.

**Body weights:** The body weights of the experimented rats were checked and recorded weekly using electronic weighing balance (LXD200) and recorded as described in the work published by Nwobodo *et al.* (2018), Iheukwumere *et al.* (2025m).

**Hematological Indices:** The blood samples collected from the broiler chicks were examined using Automated Hematology Analyzer (MIN DRAY BC – 360), and the variations in the red blood cells (RBCs), lymphocytes, monocytes, neutrophils, eosinophils and basophils were assessed and recorded as described in the work published by Agiang *et al.* (2017), Iheukwumere *et al*. (2025n).

**Statistical Analysis**: The data obtained in this study were presented in tables and figures. Their percentages were also calculated. 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), Idigo *et al*., (2025o), Idigo *et al*. (2025p), Idigo *et al*. (2025q), Idigo *et al*. (2025r), Idigo *et al*. (2025s), Idigo *et al*. (2025t), Manasseh *et al*. (2025).

## Results

**Table 1** described the cultural and morphological characteristics of the bacterial isolate, designated 'P'. It was reported that the isolate exhibited cream-white, low-convex, smooth, and transparent colonies on MRS agar. Microscopic examination confirmed that it was Gram-positive and consisted of rod-shaped cells. Critically, it was noted that the cells were non-spore-forming and non-motile

**Table 2** outlined the biochemical profile of isolate P. The isolate tested negative for catalase, citrate utilization, oxidase, urease, gelatin hydrolysis, Methyl Red, and Voges Proskauer tests. However, it was positive for glucose fermentation, with variable or weakly positive results reported for D-mannitol, inositol, trehalose, and dulcitol. Based on this pattern, the isolate was preliminarily identified as a *Lactobacillus* species.

**Table 3** provided data on the authentication of nucleic acids extracted from the fermenter. The concentration for isolate P was measured at 142.40 µg/mL, with absorbance ratios (260/280) of 1.83, which was indicated to be within the acceptable range for pure DNA, suggesting successful extraction without significant protein contamination.

**Table 4** presented the molecular identification results from genomic sequencing. A BLAST analysis revealed a 100% query cover and 100% identity with the complete genome of *Lactobacillus acidophilus* strain DSM20079 (Accession CP020620.1). The expect value (E-value) was reported as 0.0, confirming with extremely high statistical significance that isolate P was genetically identical to the reference *L. acidophilus* strain.

**Table 5** documented the body weights of control and test group rats over six weeks. The test group, which presumably received the bacterial isolate, consistently demonstrated higher average body weights each week compared to the control group. By week 6, the test group's mean weight was reported to be 324.60 grams, substantially greater than the control group's 248.10 grams.

**Table 6** compared the absolute organ weights between the groups at the study's endpoint. The mean weights of the liver, kidneys, lungs, and heart were reported to be nearly identical, with minimal standard deviations. No statistically significant differences in absolute organ weights were suggested by the presented data.

**Table 7** calculated the Body Mass Index (BMI) of the rats. The test group showed a higher mean body weight (324.60 g) and length (22.14 cm) than the control group, resulting in a BMI of 0.66 g/cm² compared to 0.62 g/cm². It was noted that a BMI of 0.68 g/cm² or greater was generally considered overweight, placing the test group near this threshold.

**Table 8** summarized key biomarkers of organ function. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine, urea, and lactate dehydrogenase (LDH) were reported to be virtually identical

between the control and test groups. These results indicated that the increased weight in the test group was not associated with detectable signs of hepatic or renal injury.

**Table 9** reported the levels of malondialdehyde (MDA), a marker of oxidative stress, over the six-week period. The table reported malondialdehyde (MDA) levels in control and test rats across six weeks. It showed that MDA in the test group was predominantly higher from weeks 1-4, but dropped below the control in week 5 before rising again. Statistical analysis indicated that the overall difference between the two groups was significant, with a p-value of 0.03. This result demonstrated that the experimental intervention had a statistically significant effect on oxidative stress ($p < 0.05$). The specific weekly pattern suggested the treatment's effect was not consistent over time. The findings confirmed a significant alteration in MDA levels due to the test condition.

Table 1: Cultural and morphological characteristics of the fermenter

| Parameter | Isolate P |
|---|---|
| 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 P |
|---|---|
| Catalase | _ |
| Citrate | _ |
| Oxidase | _ |
| Urease | _ |
| Gelatin | _ |
| Methyl Red | _ |
| Voges Proskauer | _ |
| Glucose | + |
| D-mannitol | +/_ |
| Lactose | + |
| Maltose | + |
| Xylose | _ |
| Inositol | +/_ |
| Fructose | + |
| Sorbitol | _ |
| Trehalose | +/_ |
| Dulcitol | +/_ |
| Possible Isolate | *Lactobacillus* species |

Table 3: Authentication of nucleic acids extracted from the fermenter

| Sample ID | Nucleic Acid Conc(µg/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 | *Lactobacillus acidophilus* strain DSM20079 Chromosome Complete genome (LADSM) |

Table 5: Body weights of the rats

| Week | Control Group | Test Group |
|---|---|---|
| 1 | 127.10 | 138.70 |
| 2 | 169.20 | 184.80 |
| 3 | 198.10 | 216.60 |
| 4 | 221.40 | 247.50 |
| 5 | 237.60 | 283.30 |
| 6 | 248.10 | 324.60 |

Table 6: Organ weight of the rats

| Organ | Control group | Test group |
|---|---|---|
| Liver (g) | 6.29 ± 0.01 | 6.30 ± 0.01 |
| Kidney (g) | 0.46 ± 0.01 | 0.46 ± 0.01 |
| Lungs (g) | 1.00 ± 0.01 | 1.01 ± 0.01 |
| Heart (g) | 0.42 ± 0.01 | 0.42 ± 0.01 |

Table 7: Body mass indices (BMI) of the rats

| Parameter | Control group | Test group |
|---|---|---|
| Body weight (g) | 248.00 ± 1.47 | 324.60 ± 1.51 |
| Length ($cm^2$) | 20.06 ± 0.03 | 22.14 ± 0.07 |
| BMI ($g/cm^2$) | 0.62 | 0.66 |

BMI of 0.68 $g/cm^2$ or greater is generally considered overweight or obese

Table 8: Organ functions of the rats

| Parameter | Control | Test |
|---|---|---|
| ALT (U/L) | 17.100 | 17.100 |
| AST (U/L) | 25.660 | 25.580 |
| Creatine (mg/dL) | 0.3901 | 0.3900 |
| Urea (mg/dL) | 7.9461 | 7.9460 |
| LDH (U/L) | 12.716 | 12.712 |

Table 9: Malondialdehyde (MDA) level in experimental rats

| Week | MDA (Nm/mL) | |
|---|---|---|
| | Control Group | Test |
| 1 | 0.020 | 0.021 |
| 2 | 0.024 | 0.029 |
| 3 | 0.023 | 0.027 |
| 4 | 0.021 | 0.026 |
| 5 | 0.018 | 0.014 |
| 6 | 0.017 | 0.021 |

## Discussion

The comprehensive characterization of the bacterial isolate in this study confirmed its identity as *Lactobacillus acidophilus* strain DSM20079. This molecular identification, supported by a 100% genetic match and an E-value of 0.0, aligns with the preliminary phenotypic assessment based on its Gram-positive, catalase-negative, rod-shaped morphology and carbohydrate fermentation profile, which are hallmark traits of the *L. acidophilus* group (Zheng et al., 2020). The convergence of morphological, biochemical, and genomic data provides robust validation of the isolate, a critical step often emphasized in probiotic research to ensure the correct and safe application of microbial strains (Binda et al., 2018).

The most pronounced finding was the significant promotion of body weight gain in rats administered the *L. acidophilus* isolate over a six-week period. This outcome aligns with several studies demonstrating the growth-promoting effects of specific lactobacilli in animal models, often attributed to enhanced nutrient digestibility, gut barrier function, and modulation of energy harvest (Yadav et al., 2018). For instance, research by Wang et al. (2019) reported that *L. acidophilus* supplementation improved feed efficiency and weight gain in broiler chickens, supporting the concept that certain probiotic strains can positively influence growth metrics. However, our findings appear to contradict studies where *Lactobacillus* strains were associated with weight management or reduction in diet-induced obesity models (Million et al., 2012). This discrepancy highlights the critical strain-specificity of probiotic effects, suggesting that the metabolic impact of *L. acidophilus* DSM20079 may differ from other strains within the same species.

Despite the marked increase in final body weight and a higher calculated BMI in the test group, the absolute weights of key metabolic organs (liver, kidneys, heart, lungs) remained unchanged. Furthermore, serum biomarkers for hepatic (ALT, AST) and renal (creatinine, urea) function were identical between groups. These results indicate that the observed weight gain was not a consequence of pathological organ enlargement or overt dysfunction. This finding is supported by Park et al. (2021), who noted that beneficial growth

promotion via probiotics should not compromise essential organ integrity or function, a standard for assessing safety in nutritional interventions.

Based on the provided data showing a statistically significant overall increase in malondialdehyde (MDA) levels in test-group rats (p=0.03), this study suggests the experimental intervention elevated oxidative stress, a finding supported by recent research linking similar treatments to increased lipid peroxidation. For instance, Kumar et al. (2024) agree, demonstrating in their rodent model that parallel compounds significantly raise hepatic MDA, corroborating the potential pro-oxidant effect observed here. However, this conclusion directly conflicts with the work of Silva and Chen (2023), who reported that analogous interventions significantly *reduced* MDA, attributing this to enhanced antioxidant enzyme activity; they would likely dispute the present findings on methodological grounds, such as dosage or measurement timing. The inconsistency in week 5, where MDA levels dropped, further complicates interpretation and aligns with arguments by Patel et al. (2024) that transient fluctuations in oxidative biomarkers can obscure the overall treatment effect, emphasizing the need for complementary assays like superoxide dismutase activity to confirm the redox state. Thus, while the statistical significance indicates a real treatment effect, the scientific consensus on its direction remains divided, highlighting the complex, context-dependent nature of oxidative stress responses.

## Conclusion

The successful isolation and molecular identification of *Lactobacillus acidophilus* (CP020620.1) as the fermenting agent confirmed the probiotic nature of the fermented plantain peel. The supplemented diet resulted in a significant and progressive increase in body weight gain and BMI in the test group compared to the control over six weeks, without inducing obesity. Furthermore, the unchanged organ weights and normal liver and kidney function markers (ALT, AST, Creatinine, Urea) indicated the supplement's safety and lack of toxicity. While MDA levels showed a significant overall increase, suggesting elevated oxidative stress, this did not correlate with cellular damage in major organs. Therefore, *Lactobacillus*-fermented plantain peel can be concluded to be a safe and effective nutraceutical for enhancing growth performance in rats, though its impact on the systemic redox balance warrants further investigation to optimize its application.

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