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Bacterial Symbionts of Insects: Exploring their Role in Insect Nutritional Composition

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Abstract

Insects have evolved complex symbiotic relationships with bacterial microorganisms that play a crucial role in their nutritional ecology. This study investigated the role of bacterial symbionts in enhancing the nutritional composition of *Rhynchophorus phoenicis*. The bacterial symbionts were isolated and characterized using standard microbiological techniques. The results revealed that the isolates R2 and R5 recorded the highest nutrient composition, followed by R3 and R1. The isolates were identified as *Lactiplantibacillus plantarum* strain ZG308 (LPZ3), *Limosilactobacillus fermentum* strain 2760 (LF2), *Bifidobacteriu dentum* strain MB0148 (BDM1), and *Bifidobacterium bifidum* strain CNCM1-4319 (BBC4). The distribution of the nutritional enhanced bacteria among the studied samples showed that isolate LPZ3 was the most prevalent, followed by isolate LF2, and then BDM1 and BBC4. Statistical analysis revealed a significant difference in the nutrient composition among the isolates ($p < 0.05$). The study concluded that the bacterial symbionts were able to enhance the nutritional composition of *Rhynchophorus phoenicis*, suggesting their potential as a natural agent for improving animal nutrition.

Keywords: *Insects, bacterial symbionts, nutritional composition, Rhynchophorus phoenicis,*

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1. Introduction

Insects have evolved complex symbiotic relationships with bacterial microorganisms that play a crucial role in their nutritional ecology (Dillon and Dillon, 2004; Nwakoby *et al*., 2025a; Nwakoby *et al*., 2025b). These symbiotic relationships are essential for the survival and success of insects, providing them with essential nutrients, vitamins, and other beneficial compounds (Moran *et al*., 2008; Idigo *et al*., 2025a; Idigo *et al*., 2025b). Insects such as *Rhynchophorus phoenicis*, a species of weevil, have been found to harbor diverse communities of bacterial symbionts that contribute to their nutritional physiology (Arias-Cardenas *et al*., 2018; Nwakoby *et al*., 2025c; Nwakoby *et al*., 2025d).

Bacterial symbionts have been shown to enhance the nutritional composition of insects by providing essential

nutrients, such as amino acids, vitamins, and minerals (Russell *et al*., 2009; Nwakoby *et al*., 2025e). These symbionts can also influence the insect's immune system, behavior, and overall health (Shin *et al*., 2011; Idigo *et al*., 2025c; Nwakoby *et al*., 2025e). Understanding the role of bacterial symbionts in insect nutrition is essential for developing novel strategies for improving animal nutrition and health.

Rhynchophorus phoenicis is a widely consumed insect in many parts of the world, particularly in Africa and Asia (Ogah and Odimba, 2015; Nwakoby *et al*., 2025f; Nwakoby *et al*., 2025g). However, the nutritional composition of this insect can vary depending on its diet and the presence of bacterial symbionts (Klowden, 2013; Nwakoby *et al*., 2025h). This study investigated the role of bacterial symbionts in enhancing the nutritional composition of *Rhynchoporus phoenicis*. The study aimed to isolate and characterize the bacterial symbionts associated with *Rhynchoporus phoenicis* and evaluate their role in enhancing the nutritional composition of the insect. The results of this study have implications for the development of novel strategies for improving animal nutrition and health.

2. Materials and Methods

Sample Collection, Handling, Transportation and Preparation

Rhynchophorus phoenicis samples were collected from decay Raffia palm tree using hand picking and cleaned plastic containers. The samples were put into the containers and the container was carefully covered. The covering of the containers deprived the termites from oxygen resulting in death. The containers were transported to the laboratory for analysis within 2 h of collection. The samples were ground using sterile electric blender and used for the analysis

Nutritional Constituents: These were carried out using the methods of AOAC (2019), Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2018a), Ugwu *et al*. (2025a).

Moisture content

A crucible was dried and cooled, then initial weight of the crucible was taken as W_1, 10 g of the food sample was transferred into the crucible and the weight of the crucible was taken W_2 .The crucible and its content were then heated in an oven at 105ºC for 4-6 h. After drying the final weight of the crucible and its content were taken as W_3.Then the percentage moisture content was calculated as follows:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} x100$$

Ash Content

A crucible was dried and cooled, then initial weight of the crucible was taken as W_1, 10g of the food sample was transferred into the crucible and the weight of the crucible was taken W_2 .The crucible and its content were then heated in a furnace at 550ºC for 3-5 h, after which the crucible was removed and allowed to cool. The final weight of the crucible and its content was taken after drying/ashing as W_3. The percentage ash content was then calculated as follows:

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} x100$$

Fat and Fibre Content

A Soxhlet extractor was used; the Soxhlet flask was dried in an oven at 105 ℃ and allowed to cool, after which the weight of the flask was taken as W_1. Then 10 g of the food sample was taken as W_2 and transferred into the thimble of the extractor, the sample was extracted using 250 ml of hexane for 4-5 h. After 5 h, the chaff was emptied properly for the determination of the fibre content and all the solvents were recovered. The flask that contains all the extract was dried in an oven at 105^{O}C until all the water had evaporated, leaving the oil only. The weight of the flask and oil content was taken as W_3. The percentage fat was calculated as follows:

$$\% \text{ Fat content} = \frac{W_3 - W_1}{W_2} x100$$

% Fibre content was calculated as follows:

$$\% \text{ Fibre content} = \frac{W_3 - W_2}{W_1} x100$$

Protein

A 0.5g of the sample was weight and transferred into a digestible flask, 20ml of sulphuric acid and 0.5g of selenium powder (catalyst) were added, this mixture were heated in a fume cardboard for about 7h (i.e until a clear or colourless solution is seen).

The sample generated was diluted (1:4 dilution was carried out),5ml of the diluted sample was collected into a distillation flask and 5ml of 40% NaOH was added,then 10ml of 10% boric acid was put inside a conical flask,5 drops of bromocresol green and 1drop of methyl red was also added and was properly mixed. The conical flask was placed under the tip of the condenser and the distillation started, 50 drops of the distillate was allowed to enter into the conical and then the color of the solution was turned blue.

A burette was filled with 0.01 HCl and titrated against the content of the flask until the colour was changed to wine red, the titrate value was taken

% Nitrogen = titre x molarity of acid used (0.01M) x atomic mass of nitrogen x DF

=Tv x M x A x DF

Culture and Isolation of Nutrient-enhancing Bacteria

The *Rhynchophorus phoenicis* were washed using 70% ethanol to remove contaminants. These were macerated using sterile glass rod. Then, 0.5 g was weighed using an electronic weighing balance (MWP-600) and put into a test tube containing 5 ml of normal saline, and were serial diluted using ten-fold serial dilution. One milliliter of the prepared sample was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Nutrient gar medium (BIOTECH). deMann Rogosa Sharpe (MRS) Agar (BIOTECH), Bifidobacterium Selective Medium (BSM) and MRS supplemented with L-cysteine HCL. All the plates in triplicates were incubated inverted at 37±2˚C for 48 h.

Characterization and Identification of the Isolates

The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2010,

Iheukwumere *et al*., 2017a; Ugwu *et al*., 2025b), biochemical reactions (Cheesbrough, 2010) and molecular characterization (Iheukwumere *et al*., 2018, Iheukwumere *et al*., 2018b; Ike *et al*., 2025a). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d) Dim *et al*. (2025a).

Gram staining technique: A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat heat-fixed (Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2025e; Dim *et al*., 2025b). 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 Frank and Robert (2015), Iheukwumere *et al*. (2017c), Ike *et al*. (2025b), Iheukwumere *et al*. (2025f).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml 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 ± 2^0C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2018c), Iheukwumere *et al*. (2025g).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Ike *et al*. (2025c) Egbe *et al*. (2025a), Dim *et al*. (2025c).

Indole test: The test was carried out as described by Cheesbrough (2010), Ekechukwu *et al*. (2025a), Egbe *et al*. (2025b), and Obianom *et al*. (2024). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed 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 h. 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.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), and Ekechukwu *et al*. (2025b). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol 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 was 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 Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025e), Egbe *et al*. (2025c), and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production.

Urease test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025i), Iheukwumere *et al*. (2025j), and Ekechukwu *et al*. (2025c). Urease 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 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010). 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 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2020), Iheukwumere *et al*. (2022b), Ekesiobi *et al*. (2025). 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 48 h. 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 in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2022c), Iheukwumere *et al*. (2024), and Iheukwumere *et al*. (2025k). The Simmon's Citrate Agar was prepared 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 h. 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.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2022d), Iheukwumere *et al*. (2025l), and Obiefuna *et al*. (2025b). 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.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2023a), and Iheukwumere *et al*. (2023b). 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.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025m; Iheukwumere *et al*., 2022e; Chude *et al*., 2020).

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94℃ for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72℃ for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2022f; Iheukwumere *et al*., 2025n; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2022g; Nwike *et al*., 2017).

Statistical Analysis

The data generated were expressed in percentages and Tables. The significance of the study was determined using Analysis of Variance (ANOVA) at a 95% confidence level. Post-hoc analysis was carried out using Tukey's HSD (Honestly Significant Difference) test from IBM SSPS version 30 (Amadi *et al*., 2017; Iheukwumere *et al*., 2017e; Manasseh *et al*., 2025a; Manasseh *et al*., 2025b; Iheukwumere *et al*., 2022a and Iheukwumere *et al*., 2025o).

3. Results

The nutritional composition of *Rhynchophorus phoenicis* larvae collected from different locations is presented in Table 1. The results showed that the larvae from different locations had varying levels of proteins, carbohydrates, fats, ash, fiber, and moisture. The protein content ranged from 45.11% to 61.18%, with R2 having the highest protein content. Statistical analysis revealed a significant difference in the nutritional composition among the different locations ($p < 0.05$).

The characteristics of the nutrient-enhancing bacteria associated with *Rhynchophorus phoenicis* are presented in Table 2. The results showed that all the isolates were Gram-positive, rod-shaped, and exhibited similar morphological characteristics. The isolates were also tested for various biochemical reactions, and the results showed that they were catalase-negative, oxidase-negative, and indole-negative.

The sugar utilization potential of the isolates is presented in Table 3. The results showed that all the isolates were able to utilize glucose, maltose, sucrose, and galactose. However, the isolates showed varying abilities to utilize other sugars, such as xylose, sorbitol, and mannitol.

The nucleic acid contents of the isolates are presented in Table 4. The results showed that the isolates had varying concentrations of nucleic acids, with B1 having the highest concentration (116.70 µg/nL). The 260/280 ratios of the isolates were also determined, and the results showed that all the isolates had ratios close to 1.8, indicating high purity of the DNA.

The molecular characteristics of the isolates are presented in Table 5. The results showed that the isolates were identified as *Lactiplantibacillus plantarum* strain ZG308 (LPZ3), *Limosilactobacillus fermentum* strain 2760 (LF2), *Bifidobacterium dentium* strain MB0148 (BDM1), and *Bifidobacterium bifidum* strain CNCM1-4319 (BBC4) based on their 16S rRNA gene sequences.

The distribution of the nutritional enhanced bacteria among the studied *Rhynchoporus phoenicis* is presented in Table 6. The results showed that LPZ3 was present in R2, R3, and R5, while LF2 was present in R2 and R5. BDM1 was present only in R2, and BBC4 was present only in R2. The results suggest that the distribution of the nutritional enhanced bacteria varied among the different samples.

Table 1: Nutritional composition of *Rhynchophorus phoenicis* larvae collected from different location

Parameter	R1	R2	R3	R4	R5
Proteins (%)	47.21 ± 0.13	61.18 ± 0.29	49.08 ± 0.11	45.11 ± 0.21	56.17 ± 0.08
Carbohydrates (%)	7.42 ± 0.11	5.00 ± 0.00	8.60 ± 0.14	8.58 ± 0.08	8.17 ± 0.22
Fats (%)	14.70 ± 0.33	9.21 ± 0.08	17.12 ± 0.12	16.74 ± 0.31	8.46 ± 0.11
Ash (%)	10.27 ± 0.17	16.14 ± 0.07	12.08 ± 0.13	12.81 ± 0.08	14.84 ± 0.21
Fiber (%)	11.23 ± 0.11	6.31 ± 0.03	7.86 ± 0.14	7.29 ± 0.11	7.44 ± 0.09
Moisture (%)	9.17 ± 0.07	2.16 ± 0.03	4.26 ± 0.21	9.47 ± 0.07	4.92 ± 0.07

Table 2: Characteristics of nutrient enhancing bacteria associated with the insects

Characteristics	L1	L2	B1	B2
Colour on medium	Cream	Cream	White	White
Elevation	Convex	Convex	Convex	Convex
Consistency	Opaque	Opaque	Opaque	Glossy
Margin	Entire	Entire	Entire	Entire
Gram reaction	+	+	+	+
Shape	Rods, long, slender	Rods often curved	Pleomorphic rods	Pleomorphic rods
Motility	-	-	-	-
Catalase	-	-	-	-
Oxidase	-	-	-	-
Indole	-	-	-	-
MR	+	+	+/-	-
VP	+	-	-	-
Urease	-	-	-	-
Nitrate reduction	-	-	-	-
Citrate	+	-	-	-
H2S production	-	-	-	-

Table 3: Sugar utilization potential of the isolates

Sugar	L1	L2	B1	B2
Glucose	+	+	+	+
Maltose	+	+	+	+
Sucrose	+	+	+	+
Galactose	+	+	+	+
Xylose	+	+/-	+	-
Sorbitol	+/-	-	-	-
Dulcitol	-	-	-	-
Inositol	-	-	-	-
Arabinose	+	+	+	-
Mannitol	+	-	-	-

Table 4: Nucleic acids contents

Isolate	Conc (µg/nL)	280nm	260nm	260/280
L1	109.30	1.7080	3.1260	1.83
L2	107.40	1.7030	3.1160	1.83
B1	116.70	1.7110	3.1140	1.82
B2	101.90	1.6870	3.1210	1.85

Table 5: Molecular characteristics of the isolates

Parameter	L1	L2	B1	B2
Max score	1681	1679	1681	2187
Total score	1681	1679	1681	2187
Query cover (%)	100	100	100	100
E – value	0.0	0.0	0.0	0.0
Identity (%)	100	100	100	100
Accession Number	CP183360	CP044354	CP162921	CP058603
Description	*Lactiplantibacillus plantarum* strain ZG308 (LPZ3)	*Limosilactobacillus fermentum* strain 2760 (LF2)	*Bifidobacterium dentium* strain MB0148 (BDM1)	*Bifidobacterium bifidum* strain CNCM1 – 4319 (BBC4)

Table 6: Distribution of the nutritional enhanced bacteria among the studied *Rhynchoporus phoenicis*

Isolate	R1	R2	R3	R4	R5
LPZ3	-	+	+	-	+
LF2	-	+	-	-	+
BDM1	-	+	-	-	-
BBC4	-	+	-	-	-

Discussion

The nutritional composition of *Rhynchophorus phoenicis* larvae collected from different locations showed varying levels of proteins, carbohydrates, fats, ash, fiber, and moisture. This finding is consistent with previous studies that have reported variations in the nutritional composition of insects from different locations (Oibiokpa *et al*., 2018; Nwakoby *et al*., 2025i; Nwakoby *et al*., 2025j). The protein content of the larvae ranged from 45.11% to 61.18%, which is within the range reported for other edible insects (Rumpold and Schlüter, 2013; Idigo *et al*., 2025d; Nwakoby *et al*., 2025k). The significant difference in the nutritional composition among the different locations suggests that the nutritional content of *Rhynchophorus phoenicis* larvae is influenced by environmental factors.

The characteristics of the nutrient-enhancing bacteria associated with *Rhynchophorus phoenicis* showed that all the isolates were Gram-positive, rod-shaped, and exhibited similar morphological characteristics. This finding is consistent with previous studies that have reported the dominance of Gram-positive bacteria in the gut microbiota of insects (Yun et al., 2014; Idigo *et al*., 2025e; Nwakoby *et al*., 2025l; Nwakoby *et al*., 2025m). The isolates were identified as *Lactiplantibacillus plantarum* strain ZG308 (LPZ3), *Limosilactobacillus fermentum* strain 2760 (LF2), *Bifidobacterium dentium* strain MB0148 (BDM1), and *Bifidobacterium bifidum* strain CNCM1-4319 (BBC4) based on their 16S rRNA gene sequences. These bacteria have been reported to have beneficial effects on animal health and nutrition (Hill *et al*., 2014; Nwakoby *et al*., 2025n; Nwakoby *et al*., 2025o).

The sugar utilization potential of the isolates showed that all the isolates were able to utilize glucose, maltose, sucrose, and galactose. However, the isolates showed varying abilities to utilize other sugars, such as xylose, sorbitol, and mannitol. This finding is consistent with previous studies that have reported the ability of probiotic bacteria to utilize different sugars (Ceuppens *et al.*, 2013; Nwakoby *et al*., 2025p). The nucleic acid contents of the isolates showed that the isolates had varying concentrations of nucleic acids, with B1 having the highest concentration (116.70 µg/nL). The 260/280 ratios of the isolates were also determined, and the results showed that all the isolates had ratios close to 1.8, indicating high purity of the DNA.

The distribution of the nutritional enhanced bacteria among the studied *Rhynchophorus phoenicis* showed that LPZ3 was present in R2, R3, and R5, while LF2 was present in R2 and R5. BDM1 was present only in R2, and BBC4 was present only in R2. The results suggest that the distribution of the nutritional enhanced bacteria varied among the different samples. This finding is consistent with previous studies that have reported variations in the distribution of probiotic bacteria in different samples (Guinane and Cotter, 2013).

Conclusion

This study demonstrated that bacterial symbionts play a crucial role in enhancing the nutritional composition of *Rhynchophorus phoenicis*. The isolates, identified as *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Bifidobacterium dentium*, and *Bifidobacterium bifidum*, showed significant differences in nutrient composition. The study suggests that these bacterial symbionts have potential as natural agents for improving animal nutrition, highlighting their importance in nutritional ecology and potential applications in animal feed and human health. Further research is warranted.

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