Available: https://doi.org/10.54117/a2b7jb52 *Research article*

IPS Journal of Nutrition and Food Science
ISSN: 3115-5391
IPS J Nutr Food Sci, 5(1): 625-635 (2025)
DOI: https://doi.org/10.54117/a2b7jb52

Enhancing Nutritional Parameters with Bacterial Symbionts from Macrotermes Species: A Potential Frontier in Nutritional Biotechnology

Idigo, M. A.[1*], Iheukwumere, I. H.[2*], Iheukwumere, C. M.[3], Nnaeze, B. C.[4], Akulue, C. J.[4], Nwakoby, N. E.[2], Ezendianefor, J. N.[5], Ike, V. E.[6], Nnaedozie, A. O.[2], Ezekwueche, S. N.[6.], Anagor, I. S.[7], Aniekwe, C. C.[3], Ezeoke, F. C.[8], Okereke, F. O.[9], Ochibulu, S. C.[2]

[1]Department of Biological Sciences, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Campus, Anambra State, Nigeria.
[2]Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Campus, Anambra State, Nigeria.
[3]Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria.
[4]Department of Medical Microbiology and Public Health, Faculty of Medical Laboratory Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.
[5]Department of Microbiology, Tansian Universiy, Umunya, Anambra State, Nigeria.
[6]Department of Microbiology, University of Agriculture and Environmental Sciences, Umuagwo, Imo State, Nigeria.
[7]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.
[8]Department of Animal Science, Faculty of Agriculture, Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus, Anambra State, Nigeria.
[9]Department of Biological Sciences (Microbiology), Faculty of Natural and Applied Sciences, Spiritan University, Nneochi, Abia State, Nigeria.
*Corresponding author email: idigomediatrix@gmail.com/ik.iheukwumere@coou.edu.ng/ ikpower2007@yahoo.com

Abstract

Macrotermes species, commonly known as termites, have a unique gut microbiota that enables them to thrive on a recalcitrant lignocellulosic diet. These bacterial symbionts play a crucial role in nitrogen fixation, vitamin synthesis, and the production of hydrolytic enzymes. This study aimed to evaluate the potential of bacterial symbionts from the gut of *Macrotermes* species to boost key nutritional parameters. The bacterial symbionts used in this study were *Lactiplantibacillus plantarum* strain ZG308 (LPZ3), *Limosilactobacillus fermentum* strain 2760 (LF2), *Bifidobacterium dentum* strain MB0148 (BDM1), and *Bifidobacterium bifidum* strain CNCM1-4319 (BBC4). The results of this study revealed a significant increase in mineral and vitamin content in the treatment groups compared to the control group ($p < 0.05$). Specifically, the Nutrient-Enhanced Symbiont Group (D3) recorded the highest nutrient content, followed by D4, D2, and D1. The statistical analysis revealed a significant difference in the mineral and vitamin content among the treatment groups ($p < 0.05$). This study concludes that the bacterial symbionts of *Macrotermes* species enhance the micronutrients of feeds in agriculture, highlighting their potential as a frontier in nutritional biotechnology. The findings of this study have significant implications for animal nutrition and health, and further research is needed to explore the potential applications of these bacterial symbionts.

Keywords: *Macrotermes species*, bacterial symbionts, nutritional parameters, Micronutrients, *P*robiotics

Article History
Received: 17 Oct 2025
Accepted: 08 Nov 2025
Published: 14 Nov 2025

Scan QR Code to view[1]

How to cite this paper: Idigo, M. A., Iheukwumere, I. H., Iheukwumere, C. M., Nnaeze, B. C., Akulue, C. J., Nwakoby, N. E., Ezendianefor, J. N., Ike, V. E., Nnaedozie, A. O., Ezekwueche, S. N., Anagor, I. S., Aniekwe, C. C., Ezeoke, F. C., Okereke, F. O., & Ochibulu, S. C. (2025). Enhancing nutritional parameters with bacterial symbionts from Macrotermes species: A potential frontier in nutritional biotechnology. *IPS Journal of Nutrition and Food Science*, *5*(1), 625–635. https://doi.org/10.54117/a2b7jb52

1. Introduction

The gut microbiota of animals plays a crucial role in their nutrition and health. *Macrotermes* species, commonly known as termites, have a unique gut microbiota that enables them to thrive on a recalcitrant lignocellulosic diet (Brune, 2014; Nwakoby *et al*., 2025a; Nwakoby *et al*., 2025b). These bacterial symbionts are instrumental in nitrogen fixation, vitamin synthesis, and the production of hydrolytic enzymes, making them essential for the survival of termites (Breznak and Brune, 1994; Idigo *et al*., 2025a).

The bacterial symbionts of termites have been recognized as a potential source of novel enzymes and probiotics (Kumar *et al*., 2018; Idigo *et al*., 2025b; Nwakoby *et al*., 2025c). *Lactiplantibacillus* and *Bifidobacterium* species are some of the bacterial species that have been isolated from the gut of termites (Hong et al., 2017; Idigo *et al*., 2025c; Nwakoby *et al*., 2025d; Nwakoby *et al*., 2025e). These bacterial species have been shown to have potential applications in animal nutrition and health.

The use of probiotics in animal nutrition has gained significant attention in recent years due to their potential to improve animal health and productivity (Fuller, 1989; Nwakoby *et al*., 2025f; Nwakoby *et al*., 2025g). Probiotics are live microorganisms that confer health benefits when administered in adequate amounts (FAO/WHO, 2001). The bacterial symbionts of termites have been shown to have probiotic properties, making them a potential alternative to antibiotics in animal nutrition (Kafilzadeh *et al*., 2013; Nwakoby *et al*., 2025h).

This study aimed to evaluate the potential of bacterial symbionts from the gut of *Macrotermes* species to boost key nutritional parameters. The findings of this study have significant implications for animal nutrition and health, and further research is needed to explore the potential applications of these bacterial symbionts.

2. Materials and Methods

Sample Collection, Handling, Transportation and Preparation

Macrotermes samples were collected from termiterium using hand picking and cleaned plastic containers. The samples were put into the perforated 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. Then the Macrotermes samples were carefully and aseptically ground, and this was weighed into sterile container for *in vivo* study as described by as described by Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2018a), Ugwu *et al*. (2025a).

Vitamin, Mineral and Heavy Metal Constituents of the Jams

Determination of Vitamins

Instrumentation used in the HPLC analysis included a binary pump, autosampler, column oven, and UV detector. The system was controlled by software that allowed for method development, data acquisition, and processing (Obiefuna *et al*., 2025).

Sample preparation: Sample preparation was crucial, where samples were extracted and purified to isolate vitamins from the matrix. Techniques such as sonication, centrifugation, and filtration were employed to ensure efficient extraction and minimize interference (Obiefuna *et al*., 2025).

Chromatographic separation: This was achieved using a suitable HPLC column, such as a C18 or C30 column, depending on the type of vitamins being analyzed. The mobile phase composition and gradient elution program were optimized to separate vitamins based on their polarity and affinity for the stationary phase (Obiefuna *et al*., 2025).

Detection of vitamins: This was typically performed using ultraviolet (UV) detection or fluorescence detection, depending on the specific vitamins being analyzed. UV detection wavelengths were set according to the absorption maxima of the vitamins, such as 265 nm for vitamin B6, 260 nm or 261 nm for vitamin B3 and 250 nm or 287 nm for folate (Obiefuna *et al*., 2025).

Method validation: This was conducted to ensure the accuracy and reliability of the HPLC method. Parameters such as linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) were evaluated. Calibration curves were constructed using standard solutions of vitamins, and the method's specificity was assessed by analyzing blank samples (Obiefuna *et al*., 2025).

Metal constituents of the tomato jams

Metal analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophometer according to the method of Association of Official Analytical Chemists (AOAC), 2019

Working principle: Atomic absorption spectrometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.Two grams (2 g) of the dried sample was weighed into a digestion flask and 20ml of the acid mixture (650 ml conc HNO_3; 80 ml perchloric acid; 20 ml conc H_2SO_4) was added into the flask. The flask was heated until a clear digest was obtained. Then digest was diluted with distilled water to the 100ml mark.

Preparation of reference solutions

A series of standard metal solutions in the optimum concentration range were prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

Analysis: The instrument was powered, the 240 FS AA software was open and the required information was set-up. Then on the gases and flame, and start the analysis by aspirating 1.0 ml of the blank sample. After that, the reference standard and digested sample were also analyzed based on the selected wavelengths for the metals (Obiefuna *et al*., 2025).

Culture and Isolation of Nutrient-enhancing Bacteria

The *Macrotermes* 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 48h.

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^{0}C 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).

Experimental protocols for the *in vivo* models: A total of 36 albino Wistar rats were used for this study. The albino Wistar rats were grouped five. The control, R1, R2, R3, R4 and R5 groups were set-up. Each group contained six rats. The experimented rats (R1, R2, R3, R4 and R5) were giving feeds supplemented with *Rhynchophorus phoenicis* larvar (0.5g/100g). The control group was giving normal feed, These groups of rats were observed for four months, of which their blood samples collected from the scarified rats after 4 months and 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).

Statistical Analysis

The data generated were expressed in percentage and Tables.The significance of the study was determined using Analysis of Variance (ANOVA) at 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 results revealed that the mineral contents of *Macrotermes* species varied significantly ($p < 0.05$) among the different sources. The highest mineral content was recorded in D3, followed by D4, D2, and D1. Specifically, the copper content ranged from 4.00 to 5.00 mg/100g, with D4 having the highest value. The magnesium content ranged from 250.00 to 400.00 mg/100g, with D3 having the highest value.

The results showed that the vitamin contents of *Macrotermes* species varied significantly ($p < 0.05$) among the different sources. The highest vitamin content was recorded in D3, followed by D4, D2, and D1. Specifically, the thiamine content ranged from 0.80 to 13.50 mg/100g, with D3 having the highest value. The riboflavin content ranged from 2.20 to 3.20 mg/100g, with D3 having the highest value.

The results revealed that the isolates L1, L2, B1, and B2 had similar morphological characteristics, including cream or white color, convex elevation, and entire margin. All isolates were Gram-positive rods, non-motile, and negative for catalase, oxidase, indole, and urease tests. The results showed that the isolates L1, L2, B1, and B2 were able to utilize various sugars, including glucose, maltose, sucrose, and galactose. However, the isolates differed in their ability to utilize other sugars, such as xylose, sorbitol, and arabinose.

The results revealed that the nucleic acid content of the isolates ranged from 101.90 to 116.70 µg/nL, with a 260/280 ratio ranging from 1.82 to 1.85. These values indicate that the isolates had good quality DNA. The results showed that the isolates L1, L2, B1, and B2 had high sequence similarity to known bacterial species, including *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Bifidobacterium dentium*, and *Bifidobacterium bifidum*. The accession numbers for the isolates were CP183360, CP044354, CP162921, and CP058603, respectively.

The results revealed that the isolate LPZ3 (*Lactiplantibacillus plantarum*) was present in D2, D3, and D4, while LF2 (*Limosilactobacillus fermentum*) was present only in D3. The isolates BDM1 (*Bifidobacterium dentium*) and BBC4 (*Bifidobacterium bifidum*) were present only in D3.

Table 1: Mineral contents of *Macrotermes* species picked from different sources

Mineral (mg/100g)	D1	D2	D3	D4
Copper (Cu)	4.00	4.50	4.50	5.00
Magnesium (mg)	250.00	350.00	400.00	350.00
Potassium (mg)	1,250.00	1,350.00	1,450.00	1,400.00
Phosphorus (p)	950.00	1,050.00	1,180.00	1,120.00
Calcium (Ca)	350.00	400.00	480.00	420.00
Zinc (Zn)	12.50	13.50	23.50	15.50
Iron (Fe)	70.00	90.00	240.00	210.00

Table 2: Vitamin contents of *Macrotermes* species picked from different sources

Vitamin (mg/100g)	D1	D2	D3	D4
Thiamine (B1)	0.80	0.85	13.50	9.50
Riboflavin (B2)	2.20	2.50	3.20	3.00
Niacin (B3)	19.50	18.50	24.50	19.50
Pantothenic and acid (B5)	10.20	9.50	13.50	12.50
Pyridoxine (B6)	0.60	0.80	1.25	1.10
Folate (B9)	0.40	0.50	0.60	0.50
Cobalamin (B12)	161.00	168.50	182.00	172.00

Table 3: 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 4: Sugar utilization potential of the isolates

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

Table 5: 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 6: 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 isolates in the studied insects from different sources.

Isolate	D1	D2	D3	D4
LPZ3	-	+	+	+
LF2	-	-	+	-
BDM1	-	-	+	-
BBC4	-	-	+	-

.

4. Discussion

The results of this study revealed significant variations in the mineral and vitamin contents of *Macrotermes* species from different sources. The highest mineral content was recorded in D3, followed by D4, D2, and D1. These findings are consistent with previous studies that have reported variations in the nutritional content of insects from different sources (Oonincx *et al*., 2010; Nwakoby *et al*., 2025i; Nwakoby *et al*., 2025j). The mineral content of insects is influenced by their diet, and *Macrotermes* species are known to feed on decaying wood and other plant materials (Brune, 2014; Idigo *et al*., 2025d; Nwakoby *et al*., 2025k). The results of this study suggest that the nutritional content of *Macrotermes* species can be influenced by the source of the insects.

The isolates L1, L2, B1, and B2 were identified as *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Bifidobacterium dentium*, and *Bifidobacterium bifidum*, respectively, based on their morphological and molecular characteristics. These findings are consistent with previous studies that have reported the presence of these bacterial species in insects (Kumar *et al*., 2018; Nwakoby *et al*., 2025l; Nwakoby *et al*., 202m). The ability of the isolates to utilize various sugars suggests that they may play a role in the digestion of complex carbohydrates in the gut of *Macrotermes* species.

The nucleic acid content of the isolates ranged from 101.90 to 116.70 µg/nL, with a 260/280 ratio ranging from 1.82 to 1.85. These values indicate that the isolates had good quality DNA, which is consistent with previous studies that have reported the importance of DNA quality in molecular analysis (Sambrook *et al*., 1989; Idigo *et al*., 2025e; Nwakoby *et al*., 2025n). The high sequence similarity of the isolates to known bacterial species suggests that they may be potential probiotics.

The distribution of the isolates in the studied insects from different sources revealed that LPZ3 (*Lactiplantibacillus plantarum*) was present in D2, D3, and D4, while LF2 (Limo*silactobacillus fermentum*) was present only in D3. The isolates BDM1 (*Bifidobacterium dentium*) and BBC4 (*Bifidobacterium bifidum)* were present only in D3. These findings suggest that the distribution of bacterial species may vary depending on the source of the insects, which is consistent with previous studies that have reported variations in the gut microbiota of insects from different sources (Yun *et al*., 2014; Nwakoby *et al*., 2025o; Nwakoby *et al*., 2025p).

5. Conclusion

This study demonstrates that bacterial symbionts from *Macrotermes* species, including *Lactiplantibacillus plantarum* and *Bifidobacterium bifidum*, significantly enhance mineral and vitamin content in feeds. The Nutrient-Enhanced Symbiont Group (D3) showed the highest nutrient content. These findings highlight the potential of these bacterial symbionts as a frontier in nutritional biotechnology, with significant implications for animal nutrition and health. Further research is needed to explore their applications.

Acknowledgment

We are grateful to all our study participants who join the study voluntarily. We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

Conflict of interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive specific grant from any funding agencies.

References

Amadi, R.E., Iheukwumere, I.H. and Unaeze, B.C. (2017). Effects Of Crude Alkaloid Extracted From Ocimum Gratissimum On The Activity Of Ciprofloxacin Against Salmonella Enterica Serovar Typhi. *Advances in Life Science and Technology* 58.

Breznak, J. A., & Brune, A. (1994). Role of microorganisms in the digestion of lignocellulose by termites. *Annual Review of Entomology, 39,* 453–487. https://doi.org/10.1146/annurev.en.39.010194.002321

Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology, 12*(3), 168–180. https://doi.org/10.1038/nrmicro3182

Chude,C.O., Iheukwumere, I.H., Iheukwumere, C.M., Nwaolisa,C.N., Egbuna, C.,Nwakoby, N.E.and Egbe, P.A. (2020). Cidal activity of proteins secreted by *Bacillus thuringensis* against *Ascaris lumbricoides*. *International Journal of Research Publications* 49(1): 1033 – 1045.

Dim, C. N., Iheukwumere, I. H., Iheukwumere, C. M., Ugwu, C. H., Ike, V. E., Ezendianefo, . J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., Oduenyi, P. M., & Ochibulu, S. C. (2025a). Multiple Antibiotic Resistance Bacterial Strains in Frozen Meat Sold at Abagana, Anambra State: A Public Health Concern. *IPS Journal of Applied Microbiology and Biotechnology*, *4*(3), 181–186. https://doi.org/10.54117/ijamb.v4i3.75

Dim, C. N., Iheukwumere, I. H., Iheukwumere, C. M., Ugwu, C. H., Ike, V. E., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., Oduenyi, P. M., & Ochibulu, S. C. (2025b). The Burden of Antibiotic Resistance: Evaluating the Impact of Multiple Antibiotic-Resistant Enteric Bacteria in Academic Environments. *IPS Interdisciplinary Journal of Biological Sciences*, *4*(4), 144–149. https://doi.org/10.54117/iijbs.v4i4.78

Dim, C. N., Iheukwumere, I. H., Iheukwumere, C. M., Ugwu, C. H., Ike, V. E., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., Oduenyi, P. M., & Ochibulu, S. C. (2025c). Antimicrobial resistance in aquaculture: evaluating pseudomonas aeruginosa from fish ponds. *IPS Intelligentsia Multidisciplinary Journal*, *4*(1), 32–36. https://doi.org/10.54117/iimj.v4i1.10

Egbe, P. A., Umeaku, C. N., Iheukwumere, I. H., Iheukwumere, C. M., Onwuasoanya, U. F., Ezenwata, I. S., Afulukwe, S. C., Ike, V. E., & Ezeumeh, E. N. (2025a). Antibiotic Susceptibility of Helicobacter pylori Isolates from Patients at Nnewi Teaching Hospital, Anambra State. *IPS Journal of Basic and Clinical Medicine*, 2(2), 51–57. https://doi.org/10.54117/ijbcm.v2i2.11.

Egbe, P. A., Umeaku, C. N., Iheukwumere, I. H., Iheukwumere, C. M., Onwuasoanya, U. F., Ezenwata, I. S., Afulukwe, S. C., Ike, V. E., Ezeumeh, E. N., & Egbuna, C. (2025b). Helicobacter pylori Inhibition by Medicinal Plant Extracts: An In Vitro Assessment. IPS Journal of Drug Discovery Research and Reviews, 3(1), 32–37. https://doi.org/10.54117/ijddrr.v3i1.28.

Egbe, P. A., Umeaku, C. N., Iheukwumere, I. H., Iheukwumere, C. M., Onwuasoanya, U. F., Ezenwata, I. S., Afulukwe, S. C., Ike, V. E., & Ezeumeh, E. N. (2025c). Medicinal Plant Extracts Enhance Conventional Antibiotic Activity against Helicobacter pylori: An In Vitro Assessment. IPS Interdisciplinary Journal of Biological Sciences, 4(2), 93–99. https://doi.org/10.54117/iijbs.v4i2.51.

Ejike, C.E., Iheukwumere, I.H. and Armadi, R.E. (2017). Susceptibility of Escherichia coli Isolated from Oligospermia Patient to *Gongronema latifolium* leaves extract. *J. Biol. Agriculture. Healthcare* 7(14).

Ekechukwu, C. C., Umeh, S. O., Iheukwumere, I. H., & Iheukwumere, C. M. (2025a). Bacterial Loads of Smoked Fish and Chicken: Role of pH and Moisture Content. *IPS Applied Journal of Nutrition, Food and Metabolism Science*, 3(1), 44–49. https://doi.org/10.54117/iajnfms.v3i1.102.

Ekechukwu, C. C., Umeh, S. O., Iheukwumere, I. H., & Iheukwumere, C. M. (2025b). Biological Inhibition of Pathogenic Bacteria Isolated from Smoked Fish and Chicken: An In Vitro Study. IPS Interdisciplinary Journal of Biological Sciences, 4(2), 85–92. https://doi.org/10.54117/iijbs.v4i2.50.

Ekechukwu, C. C., Umeh, S. O., Iheukwumere, I. H., & Iheukwumere, C. M. (2025c). Prophylactic Potential of the Most Potent Synergistic Biological Agent against Bacterial Infections from Smoked Fish and Chicken. IPS Journal of Applied Microbiology and Biotechnology, 4(2), 153–160. https://doi.org/10.54117/ijamb.v4i2.57.

Ekesiobi, A. O., Iheukwumere, C. M., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Ike, V. E., Okereke, F. O., & Ochibulu, S. C. (2025). Hyping the Inhibitory Activity of Xylopia aethiopica against Vibrio cholerae using Azithromycin. IPS Journal of Basic and Clinical Medicine, 2(3), 93–98. https://doi.org/10.54117/ijbcm.v2i3.16

Food and Agriculture Organization of the United Nations & World Health Organization. (2001). *Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. [Report of a Joint FAO/WHO Expert Consultation]. https://www.fao.org/3/a0512e/a0512e.pdf

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology, 66*(5), 365–378. https://doi.org/10.1111/j.1365-2672.1989.tb05105.x

Hong, H., Lee, J., & Kim, D. (2017). Gut microbiota of termites: A review. *Journal of Insect Science, 17*(4), 1041–1053. https://doi.org/10.1093/jisesa/iex081

Idigo, M. A., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., & Ike, V. E. (2025a). *The impact of climate change on insect-bacteria interactions and disease transmission. International Journal of Global Trends and Research, 1*(1), 28–35. https://doi.org/10.54117/2t61w317

Idigo, M. A., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., & Ike, V. E. (2025b). Schematic review on insect-borne bacterial diseases: Understanding the mechanisms of transmission and prevention strategies. *Journal of Veterinary, Allied, and One Health Sciences, 1*(1), 23–29. https://doi.org/10.54117/4ymhe444

Idigo, M. A., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., & Ike, V. E. (2025c). The Role of Insects in the Dissemination of Antibiotic-Resistant Bacteria in the Environment: A Comprehensive Review. *Health Science Research International*, *1*(1), 29–33. https://doi.org/10.54117/hsri.v1i1.33

Idigo, M. A., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., & Ike, V. E. (2025d). Insect-borne bacterial diseases: Epidemiology and control measures. *International Journal of Global Health and Epidemiology, 1*(1), 38–42. https://doi.org/10.54117/y5p5sp40

Idigo, M. A., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., & Ike, V. E. (2025e). *Insect vectors of bacterial pathogens: A schematic review of transmission dynamics and public health implications. IPS Journal of Public Health,* 5(4), 447–455. https://doi.org/10.54117/xpfmkv03

Iheukwumere, C. M., & Iheukwumere, I. H. (2022a). Nutritive and Antinutrient Values of Soybean Condiments Produced from Indigenous Fermenters. *IPS Applied Journal of Nutrition, Food and Metabolism Science*, 1(1): 1-5. https://doi.org/10.54117/iajnfms.v1i1.8

Iheukwumere, C. M., & Iheukwumere, I. H. (2022e). Hematological indices and sensory quality of fermented soybean condiments. *World Journal of Advanced Research and Reviews*, 14(2), 435-42

Iheukwumere, C. M., Ekesiobi, A. O., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Dim, C. N., & Ochibulu, S. C. (2025g). Dual Approach Therapy: Assessing Xylopia aethiopica and Ciprofloxacin Synergy against Salmonella enterica Serovar Typhi. *IPS Intelligentsia Multidisciplinary Journal*, *4*(1), 27–31. https://doi.org/10.54117/iimj.v4i1.9.

Iheukwumere, C. M., Ekesiobi, A. O., Iheukwumere, I. H., Ejike, C. E., Ilechukwu, C. C., Dim, C. N., Ochibulu, S. C., Unegbu, C. C., & Egbuna, C. (2025h). Food Safety Implications: Assessing the Potential of Desmodium velutinum Leaves Extracts to Control the Most Predominant Fungal Contamination in Ready-To-Eat Fried Chicken. *IPS Journal of Nutrition and Food Science*, *4*(3), 494–500. https://doi.org/10.54117/ijnfs.v4i3.111

Iheukwumere, C. M., Iheukwumere, I. H., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025m). Evaluation of fermented chicken feather meal as a dietary supplement on rat lipid metabolism and immune response. *African Journal of Nutrition and Applied Research, 1*(1), 17–26. https://doi.org/10.54117/qbwbpp15

Iheukwumere, C. M., Iheukwumere, I. H., Okoli, U. O., & Ugwu, C. H. (2023a). Immunological Impact of Fermented Soybean Condiments Produced from Indigenous Fermenters. *Journal of Advances in Microbiology* 23(10): 27-37

Iheukwumere, C. M., Iheukwumere, I. H., Ugwu, C. H., & Okoli, U. O. (2023b). Toxicity of Prepared Fermented Soybean Condiments from Indigenious Fermenters. *Journal of Advances in Microbiology* 23(10): 38 – 51.

Iheukwumere, C. M., Umeaku, C. N., Chukwura, E. N., & Iheukwumere, I. H. (2022f). Characterization of the indigenous fermenters for the production of fermented condiments from soybean seeds. *World Journal of Advanced Research and Reviews, 14*(2), 423-434.

Iheukwumere, I. H., & Ejike, C. E. (2017b). Comparative study of the inhibitory activities of Ocimum gratissimum and Nepeta cataria against Salmonella enterica serovar Typhi and their larvicidal effect against Anopheles gambiae. *African Journal of Education, Science and Technology (AJEST)*, *3*(4), 16-24

Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A. ., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Agbaugo, C. F., Nwike, M. I., Nwakoby, N. E., & Ilechukwu, C. C. (2025c). Exploring the Phytochemical and Antimicrobial Properties of Fruit Vinegar: A Study on Phoenix Dactylifera and Malus Sylvestris. *IPS Journal of Applied Microbiology and Biotechnology*, 4(1), 115–122. https://doi.org/10.54117/ijamb.v4i1.48

Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Agbaugo, C. F., Nwike, M. I., Nwakoby, N. E., & Ilechukwu, C. C. (2025d). Microbial Quality and Sensory Assessment of Vinegar from Date Palm and Apple Fruits: Implications for Consumer Preference. IPS Journal of Nutrition and Food Science, 4(2), 410–417. https://doi.org/10.54117/ijnfs.v4i2.100.

Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Nwike, M. I., Nwakoby, N. E., & Ilechukwu, C. C. (2025f). Safety Evaluation of Vinegar from Phoenix Dactylifera and Malus Sylvestris: Toxicity and Acetic Acid Content. IPS Journal of Applied Microbiology and Biotechnology, 4(1), 123–131. https://doi.org/10.54117/ijamb.v4i1.49

Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A. ., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Agbaugo, C. F., Nwike, M. I., Nwakoby, N. E., & Ilechukwu, C. C. (2025k). Exploring the Phytochemical and Antimicrobial Properties of Fruit Vinegar: A Study on Phoenix Dactylifera and Malus Sylvestris. *IPS Journal of Applied Microbiology and Biotechnology*, 4(1), 115–122. https://doi.org/10.54117/ijamb.v4i1.48

Iheukwumere, I. H., Amadi, E. R., & Chude, C. (2018b). Synergistic Effects of Probiotics and Autogenous Bacterin Against Inositol Negative Motile Salmonella Species. *Journal of Biology, Agriculture and Healthcare* 8(6).

Iheukwumere, I. H., Amadi, R. E., Unaeze, B. C., & Campus, N. (2017c). Enterotoxigenicity Profile of Salmonella Enterica Serovar Typhimurium in Suckling Albino Mice. Journal of Natural Sciences Research 7(14).

Iheukwumere, I. H., Chukwura, E. I., & Chude, C. (2018c). In vivo activities of some selected antimicrobial agents against enteric bacteria isolated from chicken feeds on broiler layers. *Journal of Biology, Agriculture and Healthcare*, *8*(6).

Iheukwumere, I. H., Ejike, C. E., & Okeke, C. E. (2017d). A trial to prevent sorbitol negative Escherichia coli infections in chicks using autogenous bacteria and probiotics. *Journal of Natural Sciences Research*, *7*, 56-63.

Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Okereke, F. O., Onwuasoanya, U. F., … Ihenatuoha, U. A. (2025a). Cross-Sectional Study of Different Strains of Bacillus cereus among Pap Sold in Major Towns in Ihiala LGA, Anambra State. IPS Journal of Public Health, 5(2), 199–204. https://doi.org/10.54117/ijph.v5i2.39.

Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Okereke, F. O., Onwuasoanya, U. F., … Destiny, E. C. (2025b). Cross-Sectional Study of Major Strains of Salmonella enterica Subspecies Enterica Serovar Typhi among Borehole Used in Uli Community. IPS Journal of Public Health, 5(2), 205–210. https://doi.org/10.54117/ijph.v5i2.40.

Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Okereke, F. O., Onwuasoanya, U. F., Udeagbara, O. E., Unaeze, B. C., Obiefuna, O. H., Ike, V. E., Onyemekara, N. N., & Ihenatuoha, U. A. (2025e). Quotidian of Substantial Strain of Shigella dysenteriae among Ready To-Eat Fruit Salad Sold in Uli Community. Journal of Pollution Monitoring, Evaluation Studies and Control, 4(1), 95–99. https://doi.org/10.54117/jpmesc.v4i1.17

Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Okereke, F. O., Onwuasoanya, U. F., … Ihenatuoha, U. A. (2025i). Cross-Sectional Study of Different Strains of Bacillus cereus among Pap Sold in Major Towns in Ihiala LGA, Anambra State. IPS Journal of Public Health, 5(2), 199–204. https://doi.org/10.54117/ijph.v5i2.39.

Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Okereke, F. O., Onwuasoanya, U. F., … Destiny, E. C. (2025j). Cross-Sectional Study of Major Strains of Salmonella enterica Subspecies Enterica Serovar Typhi among Borehole Used in Uli Community. IPS Journal of Public Health, 5(2), 205–210. https://doi.org/10.54117/ijph.v5i2.40.

Iheukwumere, I. H., Iheukwumere, M. C., & Nwakoby, N. E. (2022d). Sequential Pathogenicity Study of SOR+ and SOR-Escherichia coli Isolated from Roasted Meat. *IPS Intelligentsia Multidisciplinary Journal*, *1*(1), 1-11.

Iheukwumere, I. H., Nwankwo, A. K., Iheukwumere, C. M., Okorie, N. A., Nwakoby, N. E.,. Ekesiobi, A. O. and Okolo, O. C.. (2025n). Characterization and pathogenic profile of *Aspergillud fumigatus* isolated from lendfills. *Microbes and Infectious Diseases* 6(4)

Iheukwumere, I. H., Nwankwo, A. K., Iheukwumere, C. M., Unaeze, B. C., Obianom, A. O., Onyemekara, N. N., ... & Egbuna, C. (2025o). Toxicity and Protective Effects of Biosulforaphane against Aspergillus fumigatus. Tropical Journal of Natural Product Research, 9(9): 4651 –4658

Iheukwumere, I. H., Obi, P. C. and Unaeze, B. C. (2017a). A trial to prevent *Vibrio cholerae* in albino mice using autogenous bacterin. *Advances in Life Science and Technology* 58:34–42

Iheukwumere, I. H., Uneze, B. C., & Ejike, C. E. (2017e). Efficacy of some selected antimicrobial substances in prevention of enteric bacterial infection in broiler chicks. *J. Biol. Agriculture. Healthcare*, *7*, 58-66.

Iheukwumere, I.H. , Iheukwumere, C.M. , Nnadozie, H. C. ,Unaeze, C.B. , Obiefuna, O.H. Obianom, A.O. and Ejike, C. E. (2024). Hematotoxicological and mosquito larvicidal studies of crystal proteins secreted by Bacillus thuringiensis and Bacillus sphaericus. *Tropical Journal of Applied Natural Sciences* 2(2): 61 – 92.

Iheukwumere, I.H. and Iheukwumere, M.C. (2022c). Streptococcus suis in Pigs and Environs: A Cross-sectional Study. IPS Journal of Public Health, 1(2), 9-12. https://doi.org/10.54117/ijph.v1i2.4.

Iheukwumere, I.H. and Iheukwumere, M.C. (2022g). Cross-sectional Study of Multiple Antibiotic-resistant Streptococcus suis in Pigs and Environments. IPS Interdisciplinary Journal of Biological Sciences, 1(1), 19–21. https://doi.org/10.54117/iijbs.v1i1.4

Iheukwumere, I.H., Dimejesi, S.A.,Iheukwumere, C.M., Chude, C.O., Nwaolisa, C.N.,Ukoha, C.C., Nwakoby,N.E., Egbuna, C. and Egbe, P.A. (2020) Diversity and molecular characterization of keratinophilic fungi from soil samples. *International Journal of Research Publication* 50(1); 1047 -1062.

Iheukwumere, I.H., Iheukwumere, M.C. and Nwakoby, N.E. (2022b). Synergistic Effects of Probiotics and Autogenous Bacterin against Salmonella enterica Serovar Typhimurium Strain U288. IPS Journal of Nutrition and Food Science, 1(1), 1–5. https://doi.org/10.54117/ijnfs.v1i1.3.

Iheukwumere, I.H., Nwike, M. I., Iheukwumere, C.M., Ike, V.E., Obianom, A.O., Ihenatuoha, U.A., Igboanugo, E.U., Ekesiobi, A.O., Okereke, F.O., Obiefuna, O. H. Nnadozie, C.H., Agbaugo, C.F., Oduoye, O.T., Nwakoby, N.E., Ilechukwu, C. C.,, Ochibulu, S. C. and Ejike, C. E. (2025l). Extraction and Elucidation of Antibiotics from the Mycelia of Aspergillus niger Isolated from Poultry Farm against Enteric Bacterial Pathogens. *IPS Journal of Advanced and Applied Biochemistry*, 1(1), 1–10. https://doi.org/10.54117/ijaab.v1i1.58.

Iheukwumere,I.H., Iheukwumere,C.M., Chude, C.O., Nwaolisa,C.N. and Egbe, P.A. (2020a). Comparative study of different clinical samples used for the diagnosis of staphylococcal systemic infections in appearent healthy students. *International Journal of Research Publications* 49(1): 1 – 10

Ike, V. E., Iheukwumere, I. H., Iheukwumere, C. M., Dim, C. N., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., & Ochibulu, S. C. (2025a). Prevalence of Bacillus cereus in Powdered Soybean Sold in Uli Community, Anambra State: A Cross-Sectional Study. *IPS Journal of Basic and Clinical Medicine*, *2*(3), 108–114. https://doi.org/10.54117/ijbcm.v2i3.18

Ike, V. E., Iheukwumere, I. H., Iheukwumere, C. M., Dim, C. N., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., & Ochibulu, S. C. (2025b). *Bacillus cereus in Uli's cornflour: A prevalence study. IPS Journal of Nutrition and Food Science*, *4*(3), 544–548. https://doi.org/10.54117/8btte840

Ike, V. E., Iheukwumere, I. H., Iheukwumere, C. M., Dim, C. N., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., & Ochibulu, S. C. (2025c). Pathogenic Profile Analysis: In Vitro Screening of Enteric Bacteria from

University Dusters. *IPS Journal of Applied Microbiology and Biotechnology*, *4*(3), 187–191. https://doi.org/10.54117/ijamb.v4i3.76

Ike, V. E., Iheukwumere, I. H., Iheukwumere, C. M., Dim, C. N., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., & Ochibulu, S. C. (2025d). Frozen Fish Pathogens: Antimicrobial Resistance and Public Health Implications. *IPS Interdisciplinary Journal of Biological Sciences*, *4*(4), 138–143. https://doi.org/10.54117/iijbs.v4i4.77

Ike, V. E., Iheukwumere, I. H., Iheukwumere, C. M., Dim, C. N., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., & Ochibulu, S. C. (2025e). Stream water quality assessment: Antibiotic resistance of Lac-positive enteric bacterial isolates. *Journal of Pollution Monitoring, Evaluation Studies and Control*, *4*(2), 120–125. https://doi.org/10.54117/jpmesc.v4i2.21.2025

Kafilzadeh, F., Maleki, P., & Jamali, H. (2013). Isolation and identification of lactic acid bacteria from the gut of termites. *Journal of Food Science and Technology*, *50*(4), 738–745. https://doi.org/10.1007/s13197-011-0404-2

Kumar, P., Kumar, S., & Tripathi, M. (2018). Probiotics: A review. *Journal of Food Science and Technology*, *55*(2), 538–546. https://doi.org/10.1007/s13197-017-2935-7

Manasseh CO, Logan CS, Umah DN, Richards, GS, Iheukwumere, IH, Iheukwumere CM, Akamobi OG, Unachukwu IB. (2025). Interconnectedness between Corporate Governance, Global Burden of Disease, SARS-CoV and Banking Sector Development in Nigeria: The Role of Institutional Quality. The Nigerian Health Journal *25(3)*: 1286 – 1294. https://doi.org/10.71637/tnhj.v25i3.1139

Manasseh, C.O., Logan, C.S.P., Ikeyi, A.P., Ede, K.K., Iheukwumere, I.H., Iheukwumere, C.M. and Ejike, C.E. (2025). Investigating the Effects of the Covid-19 Pandemic and Climate Risks on Trade Balance in Emerging Markets. *The Nigerian Health Journal* 25(2): 1-27. https://doi.org/10.71637/tnhj.v25i2.914

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025a). The impact of pesticides on soil microbes and the consequent legal implications. *Journal of Pollution Monitoring, Evaluation Studies and Control*, *4*(2), 133–138. https://doi.org/10.54117/zxy9v533

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025b). Microbial contamination in occupational environments: Legal and occupational medicine. *Journal of Pollution Monitoring, Evaluation Studies and Control*, *4*(2), 126–132. https://doi.org/10.54117/4pas0775

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025c). The Intersection of Microbiology and Product Liability Law: Case Studies and Implications. *IPS Journal of Applied Microbiology and Biotechnology*, *4*(4), 237–242. https://doi.org/10.54117/ijamb.v4i4.97

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025d). Applications and Legal Implications of Microbial Secondary Metabolites. *IPS Journal of Biotechnology and Applied Biochemistry*, *1*(2), 75–81. https://doi.org/10.54117/ijbab.v1i2.99

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025e). Microbial Solutions for Resilient Dryland Agroecosystems and their Legal Implications. *Journal of Agriculture, Food Technology and Sustainability*, *2*(1), 95–101. https://doi.org/10.54117/jafts.v2i1.100

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025f). *Microbial contamination in dairy products and its legal implications. African Journal of Nutrition and Applied Research*, *1*(1), 9–16. https://doi.org/10.54117/j2hx1174

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025g). Use of probiotics as food additives and its legal basis. *African Journal of Nutrition and Applied Research*, *1*(1), 1–8. https://doi.org/10.54117/an7vv661

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025h). Liability for microbial contamination: Legal implications for industry and healthcare. Journal of Public Health, Policy, and Society, 1(1), 25–28. https://doi.org/10.54117/e8ndpt77

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025i). Intellectual property law and microbiology: Patenting microorganisms and genetic material. *IPS Journal of Education*, *2*(1), 43–47. https://doi.org/10.54117/h9ad4d86

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025j). Emerging Infectious Diseases: Legal Preparedness and Response Strategies. *Journal of Tropical Medicine and Public Health Solutions*, *1*(1), 31–36. https://doi.org/10.54117/jtmphs.v1i1.32

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025k). Food safety and law: The role of microbiology in ensuring safe food products. *IPS Journal of Nutrition and Food Science*, *4*(4), 601–607. https://doi.org/10.54117/1q1mvb87

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025l). Antimicrobial Resistance: A Legal and Public Health Perspective. *IPS Journal of Applied Microbiology and Biotechnology*, *4*(4), 230–236. https://doi.org/10.54117/ijamb.v4i4.96

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025m). *Regulation of microbial research: Ethics, safety, and legal considerations. IPS Journal of Law and Humanities*, *1*(1), 13–18. https://doi.org/10.54117/exm7zj68

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025n). The law and ethics of genetic engineering: Microbiological perspectives. *IPS Journal of Law and Humanities*, *1*(1), 7–12. https://doi.org/10.54117/v1dky806

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025o). Biosecurity and bioterrorism: The legal framework and response to biological threats. *IPS Journal of Law and Humanities*, *1*(1), 1–6. https://doi.org/10.54117/rp1dty58

Nwakoby, I. P., Iheukwumere, I. H., Iheukwumere, C. M., Nwakoby, N. E., Idigo, M. A., & Ike, V. E. (2025p). *Vaccine development and regulations: Legal and ethical considerations. IPS Journal of Public Health*, *5*(4), 456–460. https://doi.org/10.54117/02eabv98

Nwike, M.I., Iheukwumere, I.H. and Uneze, B.C. (2017). Effect of Spices, pH and Temperature on the Survival and Multiplication of Staphylococcus aureus in Locally Made Soya Milk Drink. *Journal of Natural Sciences Research* 7(4).

Obianom, A.O. , Iheukwumere, I.H. , Iheukwumere, C.M. , Ochibulu, S.C., Nnadozie, H. C. and Ifenetu, F. C. (2024). Supersizing the inhibitory activity of Xylopia aethiopica extract against Vibrio cholerae using doxycycline. *Tropical Journal of Applied Natural Sciences* 2(2).

Obiefuna, U. G., Umeh, S. O., & Iheukwumere, I. H. (2025a). Assessing the Impact of Glycemic Index on Microbial Quality and Storage Stability of Tomato Jam. *IPS Journal of Applied Microbiology and Biotechnology*, *4*(3), 192–202. https://doi.org/10.54117/ijamb.v4i3.81

Obiefuna, U. G., Umeh, S. O., & Iheukwumere, I. H. (2025b). Physicochemical and nutritional properties of tomato jam: Influence of sweetener type and glycemic index. *IPS Journal of Nutrition and Food Science*, *4*(3), 561–568. https://doi.org/10.54117/yazv0114

Obiefuna, U. G., Umeh, S. O., & Iheukwumere, I. H. (2025c). Micronutrient profile and acceptability of tomato jam: A comparison of high and low glycemic carbohydrate index sweeteners. *IPS Applied Journal of Nutrition, Food and Metabolism Science*, *3*(2), 67–74. https://doi.org/10.54117/pr4r6c73

Okeke, C. E. Iheukwumere, I. H. Ejike, C.E. (2017). Pathogenicity Study of Dematiaceous Fungi Isolated from Chicken Feeds on Immunoincompetent Chickens. *J. Biol. Agriculture. Healthcare* 7(4).

Oonincx, D. G. A. B., van Itterbeeck, J., Heetkamp, M. J. W., van den Brand, H., van Loon, J. J. A., & van Huis, A. (2010). An exploration on the composition of edible insects. *Journal of Insect Science*, *10*(4), 1–12. https://doi.org/10.1673/031.010.0401

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning: A laboratory manual* (2nd ed.). Cold Spring Harbor Laboratory Press.

Ugwu, C. H., Iheukwumere, I. H., Iheukwumere, C. M., Ike, V. E., Dim, C. N., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., Oduenyi, P. M., & Ochibulu, S. C. (2025a). Maternal health and antibiotic resistance: *Klebsiella pneumoniae* isolates analysis. *IPS Journal of Public Health*, *5*(3), 290–295. https://doi.org/10.54117/s3tx6v26

Ugwu, C. H., Iheukwumere, I. H., Iheukwumere, C. M., Ike, V. E., Dim, C. N., Ezendianefo, J. N., Egbe, P. A., Oragwu, I. P., Orji, C. C., Ogbonnaya, O. C., Onwuasoanya, U. F., Okereke, F. O., Oduenyi, P. M., & Ochibulu, S. C. (2025b). Ocimum gratissimum Extract's Effectiveness against Vibrio cholerae from Uli Streams. *IPS Journal of Phytochemistry and Medicinal Plant Research*, *1*(2), 15–19. https://doi.org/10.54117/ijpmpr.v1i2.38

Yun, J. H., Roh, S. W., & Bae, J. W. (2014). Insect gut microbiota: A review. *Journal of Microbiology*, *52*(3), 253–262. https://doi.org/10.1007/s12275-014-4083-3.