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Phylogenetic identification of microbes from fermented botanicals used in gluten-free composite flour mixes

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Abstract:
Phylogenetic information on microbial communities involved in fermenting botanicals has important implications for the food industry since it can provide a valuable perspective on the diversity, composition, and techno-functional properties and characteristics of the final product. Microbial phylogenetic analysis illustrates the evolutionary history of microbes through visual representational graphs (phylogenetic trees) showing the beginning and advancement of their assemblage.
In this study, we used molecular methods to determine the phylogenetic identities of microbes occurring in spontaneously fermented sweet potato, maize, and pigeon pea samples after a 72-hourly evaluation every 12 h. The sequences obtained were edited using the bioinformatics algorithm against similar sequences downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN and aligned using ClustalX. The neighbor-joining technique was applied to extrapolate the chronicle of the isolates evolution.
Molecular identification from the BLASTN results showed the following bacterial isolates: *Lysinibacillus macrolides*, *Klebsiella pneumoniae*, *Lactococcus lactis*, *Providencia stuartii*, *Enterobacter cloacae*, *Limosilactobacillus fermentum*, *Lactobacillus fermentum*, *Staphylococcus edaphicus*, and *Bacillus flexus*, as well as the following fungal isolates: *Trichosporon asahii*, *Mucor irregularis*, *Cladosporium tenuissimum*, and *Aspergillus niger*. The sequences obtained from the isolates produced an exact match with the NCBI non-redundant nucleotide (nr/nt) database. *L. lactis* had the highest percentage occurrence for bacteria (38.46%), while *T. asahii* and *A. niger* showed the highest occurrence for fungi (37.50%).
Identifying and characterizing the microorganisms involved in the fermentation process would allow optimizing fermentation conditions to enhance the quality and nutritional value of the final products.

Keywords: Phylogenetic identification, fermented botanicals, gluten-free composite, flour mixes

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INTRODUCTION

Microbial fermentation is a bio-engineered process that has been employed for centuries in the production of various nutrient-enhancing fermented food products such as beverages, bread, and dairy foods [1–5]. During this procedure, microorganisms such as bacteria and fungi enzymatically break down complex organic compounds inherent in the food substrate to produce various metabolites, including organic acids, alcohols, and gases [6]. Microbial communities involved in fermentation are diverse and complex, and their compositions and activities are influenced by various factors such as temperature, pH, and the presence of nutrients [7–9].

In recent years, there has been a growing interest in the use of botanicals as a substrate for microbial fermentation, particularly in the production of traditional fermented foods and beverages. The main purpose is to develop agro-processed, highly-nutritive, gluten-free therapeutic foods from comparatively advantageous indigenous botanicals [10–12]. Such botanicals include various plant materials (grains, vegetables, fruits, roots, and tubers) that are rich in nutrients and bioactive compounds and

can influence the growth and metabolism of microorganisms [13]. Studies of microbial communities involved in the fermentation of botanicals have important implications for the food industry, as they can provide insights into the techno-functional properties and characteristics of the final product [12].

Customary methods for microbial characterization have been challenged overtime due to imprecise and ambiguous similarities they provide between different species [14]. This irregularity in morphological nomenclature for microorganisms necessitated a need for modern, more pragmatic and reliable taxonomic protocols. Phylogenetics is a molecular-based technique with an improved and more proficient method of characterizing and identifying microorganisms [15]. It entails studying progressive evolutionary relationships among organisms based on their genetic characteristics from a similar forebear [16]. This powerful tool for understanding the diversity and structure of microbial communities has become a ubiquitous part of biological analyses [17, 18].

Phylogenetic analysis of microbial isolates is one of the means by which one can learn about the evolutionary history of species by constructing comparative visual representational graphs of phylogenetic trees (illustrations showing the beginning and advancement of assemblage of organisms) using the organism's morphological features [15, 19]. Recently, sequences from deoxyribonucleic acid (DNA) and proteins from different organisms have been used to determine their evolutionary relationships from common forebears to different off-springs [16, 20, 21].

Studying phylogenetic properties of microbial isolates is crucial in understanding the evolutionary history and diversity of microbial populations. Their evolutionary relationships provide insights into the origins, diversification, and distribution of microbial species. Phylogenetic properties can provide a better understanding of the microbial world, its interactions with the environment, and its impact on human health and disease [22]. This knowledge can be applied in various fields, such as biotechnology, medicine, and agriculture, to develop innovative solutions to tackle the challenges we face today [23].

In this study, we aimed to phylogenetically identify microorganisms isolated from fermented botanicals used to formulate gluten-free composite flour mixes.

STUDY OBJECTS AND METHODS

Collection and confirmation of samples. Yellow-fleshed sweet potato tubers (*Ipomea batatas* L.), maize of the yellow-grain variety (*Zea mays* L.), and pigeon peas (*Cajanus cajan* (L.) Millsp.) were obtained from local food merchants in the Auchi metropolis, specifically in the Etsako-West Local Government Area of Edo State, Nigeria. The authenticity and quality of these samples were verified at the Herbarium Curation Division, Department of Basic Sciences, Edo University Uzairue, also located in Edo State, Nigeria.

Preparation and fermentation of samples. The samples of botanical materials were subjected to fermentation, which was carried out spontaneously for 72 h. The process took place at 28 ± 2°C, following the procedures described in [12].

Microbiological analysis. The fermented botanical samples underwent microbiological analysis every 12 h to determine the total microbial counts. The method used for this analysis followed the guidelines provided by the American Public Health Association [24, 25]. To initiate the analysis, 1 mL of the fermented samples was aseptically withdrawn and mixed with 9 mL of peptone water. Subsequently, we performed a sequential 10-fold dilution.

For the microbiological analysis, aliquots from the final dilutions were taken and introduced into specific agar media. Bacteria were cultured using Nutrient Agar (NA), MacConkey Agar (MCA), and De Mann-Rogosa-Sharpe Agar (MRS). Fungi, on the other hand, were cultured using Potato Dextrose Agar (PDA). The plates containing the samples and agar media were then incubated for 24–48 h at 37°C for bacteria and at room temperature (25 ± 2°C) for fungi.

All of these processes, including dilution, culturing, and incubation, were conducted in triplicate to ensure accuracy and reliability of the results.

Isolation and enumeration of bacteria and fungi. Distinct colonies with varying morphologies were counted and reported as colony-forming units per milliliter (CFU/mL) of the respective samples. To classify these colonies, they were separated as pure cultures and preserved in agar slants both at 4°C and at room temperature. Standard morphological, biochemical, and molecular techniques were employed to confirm the identification of the different bacterial and fungal species [26, 27].

Molecular identification. ***Bacterial genomic DNA extraction.*** Five milliliters of an overnight liquid culture of the bacterial isolate in Luria Bertani (LB) medium was centrifuged at 14 000 rpm for 3 min. The resulting pellet was then resuspended in 500 mL of normal saline and heated at 95°C for 20 min. After cooling on ice, the mixture was spun for another 3 min at 14 000 rpm. The resulting supernatant, which contained the DNA, was carefully removed and transferred to a 1.5-mL micro centrifuge tube. This DNA extract was then stored at –20°C for future downstream reactions.

DNA quantification. The genomic DNA obtained was measured for its quantity using a Nanodrop 1000 spectrophotometer. To initiate the process, the Nanodrop software was opened by double-clicking on the Nanodrop icon. The spectrophotometer was calibrated using 2 µL of sterile distilled water as the initial blank, which was replaced with normal saline. Next, 2 µL of the extracted DNA was carefully loaded onto the lower pedestal of the spectrophotometer, and the upper pedestal was lowered to allow contact with the DNA sample. When the "measure" button was clicked, the Nanodrop spectrophotometer provided the measurement of the DNA concentration.

16S rRNA amplification. The 16S ribosomal RNA (rRNA) region of the rRNA genes in the isolates was amplified using the 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3')

primers. The amplification process was carried out in a 50 µL final volume for 35 cycles, using an ABI 9700 Applied Biosystems thermal cycler. The PCR mix consisted of the X2 Dream Taq Master Mix provided by Inqaba, South Africa, which included taq polymerase, DNTPs, and MgCl, along with the primers at a concentration of 0.4 M. The extracted DNA served as a template for the PCR reaction. The PCR reaction conditions were as follows: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 30 s, which was repeated for 35 cycles, and final extension at 72°C for 5 min. The resulting PCR product was separated on 1% agarose gel, subjected to electrophoresis at 120V for 15 min, and visualized using a UV transilluminator.

Fungal genomic DNA extraction. DNA extraction was performed using a ZR fungal DNA mini prep extraction kit provided by Inqaba, South Africa. Fungal isolates in pure culture were densely grown and suspended in 200 µL of isotonic buffer, to be then transferred to ZR Bashing Bead Lysis tubes. To this, 750 µL of lysis solution was added, and the tubes were placed securely in a bead beater equipped with a 2-mL tube holder assembly. The samples underwent processing at maximum speed for 5 min.

Subsequently, the ZR Bashing Bead Lysis tubes were centrifuged at 10 000 × g for 1 min. After centrifugation, 400 µL of the supernatant was carefully transferred to a Zymo-Spin IV spin Filter (orange top) positioned in a collection tube. The collection tube was then centrifuged at 7000 × g per 1 min. Then, 1200 µL of fungal/bacterial DNA binding buffer was added to the filtered liquid in the collection tube, resulting in a final volume of 1600 µL. Next, 800 µL of this mixture was moved to a Zymo-Spin IIC column placed in the collection tube, which was centrifuged at 10 000 × g for 1 min. The flow-through was discarded, and the remaining volume was retained within the Zymo-Spin IIC column.

Then, 200 µL of DNA pre-wash buffer was added to the Zymo-Spin IIC column in a fresh collection tube and centrifuged at 10 000 × g for 1 min. Following this, 500 µL of fungal/bacterial DNA wash buffer was added to the column, and centrifugation was performed at 10 000 × g for 1 min. The Zymo-Spin IIC column was then transferred to a clean 1.5-µL centrifuge tube. To elute the DNA, 100 µL of DNA elution buffer was added to the column matrix and centrifuged at 10 000 × g for 30 s. The resulting DNA, which was of high purity, was stored at –20°C for subsequent downstream reactions.

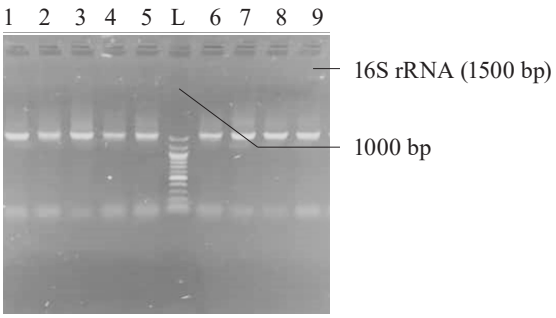
Internal Transcribed Spacer (ITS) amplification. The ITS region of the rRNA genes present in the fungal isolates was amplified using specific primers, namely ITS1-F: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4-R: 5'-TCCTCCGCTTATTGATATGC-3'. This amplification process was carried out on an ABI 9700 Applied Biosystems thermal cycler, with a final reaction volume of 50 µL for a total of 35 cycles. The PCR mixture consisted of the X2 Dream Taq Master Mix provided by Inqaba, South Africa, which contained taq polymerase, DNTPs, and MgCl, along with the primers at a concentration of 0.4 M. The extracted DNA served as a template for the PCR reaction. The PCR conditions involved initial denaturation at 95°C for 5 min, followed by denaturation at 95°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 30 s. These steps were repeated for 35 cycles, and a final extension was performed at 72°C for 5 min. To visualize the PCR product, it was separated on a 1% agarose gel using an electric field of 120V for 15 min, and the resulting bands were observed under a UV transilluminator.

Sequencing was conducted at Inqaba Biotechnological in Pretoria, South Africa, using the BigDye Terminator Kit on a 3510 ABI sequencer. The sequencing reaction was prepared with a final volume of 10 µL, consisting of the following components: 0.25 µL of BigDye® Terminator v1.1/v3.1, 2.25 µL of 5× BigDye sequencing buffer, 10 micromolar Primer PCR primer, and 2–10 nanograms of PCR template per 100 base pairs. The sequencing process involved 32 cycles with the following temperature conditions: denaturation at 96°C for 10 s, annealing at 55°C for 5 s, and extension at 60°C for 4 min.

Phylogenetic analysis of the isolates. The obtained sequences underwent editing using the bioinformatics algorithm Trace Edit. To identify similar sequences, we used the National Center for Biotechnology Information (NCBI) database. These sequences were then aligned using ClustalX. The evolutionary history of the isolates was inferred using the neighbor-joining technique [28]. The resulting phylogenetic tree displayed the most supported relationships, with a branch length summation of 0.73390024. To assess the reliability of the tree, a bootstrap test with 1000 replicates was conducted, showing the percentage of replicate trees in which the taxa were grouped together near the branches [29]. The tree was visualized with branch lengths reflecting the inferred evolutionary distances. The phylogenetic analysis involved the computation of the phylogenetic space using the Jukes-Cantor procedure, considering 17 nucleotide progressions [30]. Sites with less than 95% inclusion were removed, representing less than 5% of the sequence space, and positions allowing for cryptic bases were retained using a partial removal selection approach. The final dataset consisted of 324 positions. The evolutionary analysis was performed using MEGA X [31].

RESULTS AND DISCUSSION

Molecular identification from the BLASTN results of the DNA sequences shows that the 16S rRNA sequence obtained from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database (Fig. 1). The 16S rRNA of the isolates W1 showed the 99–100 % percentage similarity to other species. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Providencia*, *Enterobacter*, *Klebsiella*, *Lysinibacillus*, *Staphylococcus*, *Limosilactobacillus*, *Lactobacillus*, and

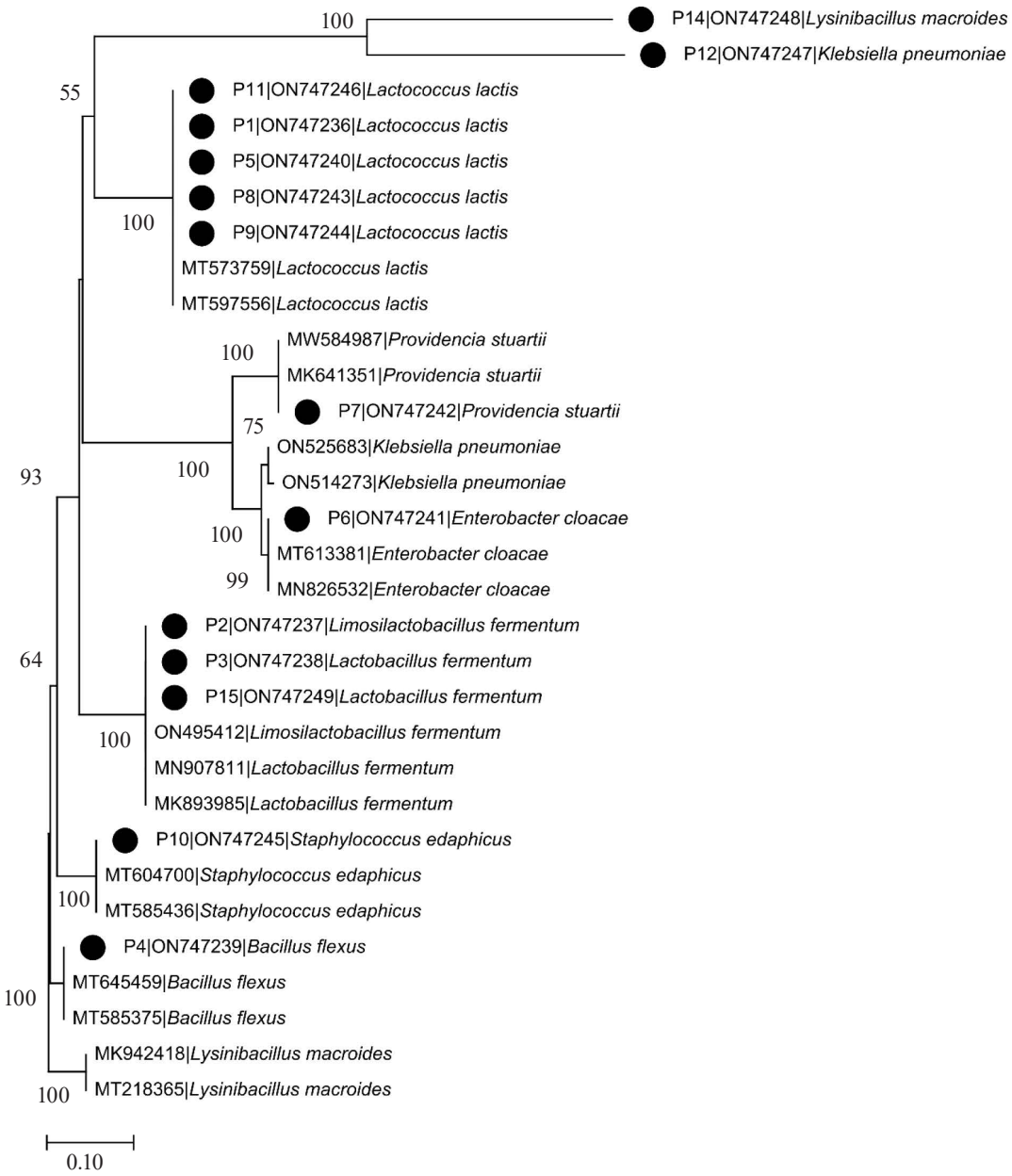


Note: lanes 1–9 represent the 16S rRNA bands at 1500 bp, while lane L represents the 100 bp molecular ladder

Figure 1 Agarose gel electrophoresis of the 16S rRNA of the bacterial isolates.

Lactococcus sp. They also revealed a close relatedness to *Providencia stuartii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Lysinibacillus macroides*, *Staphylococcus edaphicus*, *Limosilactobacillus fermentum*, *Lactobacillus fermentum*, and *Lactococcus lactis* (Fig. 2).

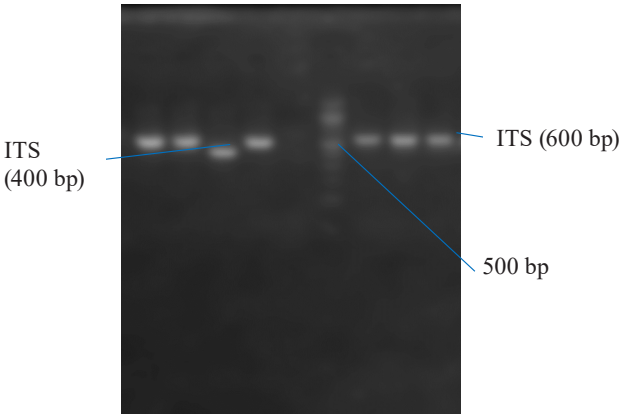
The ITS sequence obtained from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database (Fig. 3). The ITS of the isolates showed the 100% percentage similarity to other species. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement within the *Aspergillus*, *Cladosporium*, *Mucor*, and *Trichosporon* sp., as well as revealed a close relatedness to *Aspergillus niger*, *Cladosporium tenuissimum*,



Note: The nodes without circular annotation represent highly similar species that were obtained as top hits from the NCBI (National Centre for Biotechnology Information)

Figure 2 A phylogenetic tree showing the classification and evolutionary relationship of the bacterial isolates

Mucor irregularis, and *Trichosporon asahii* (Fig. 4). The morphological characteristics of the identified microbial and fungal isolates, which help in authenticating these isolates, are presented in Tables 1 and 3, respectively, while their actual names and the summary of their closest BLASTN similarities are shown in Tables 2 and 4, respectively. The percent similarities between the isolated microorganisms and those from the GenBank database indicate that they all share a common ancestry [15].



Note: lanes 1–4 and 5–8 represent the ITS bands at 400 bp and 600 bp, while lane L represents the 100 bp molecular ladder

Figure 3 Agarose gel electrophoresis showing the amplified ITS fragment of the fungal isolates

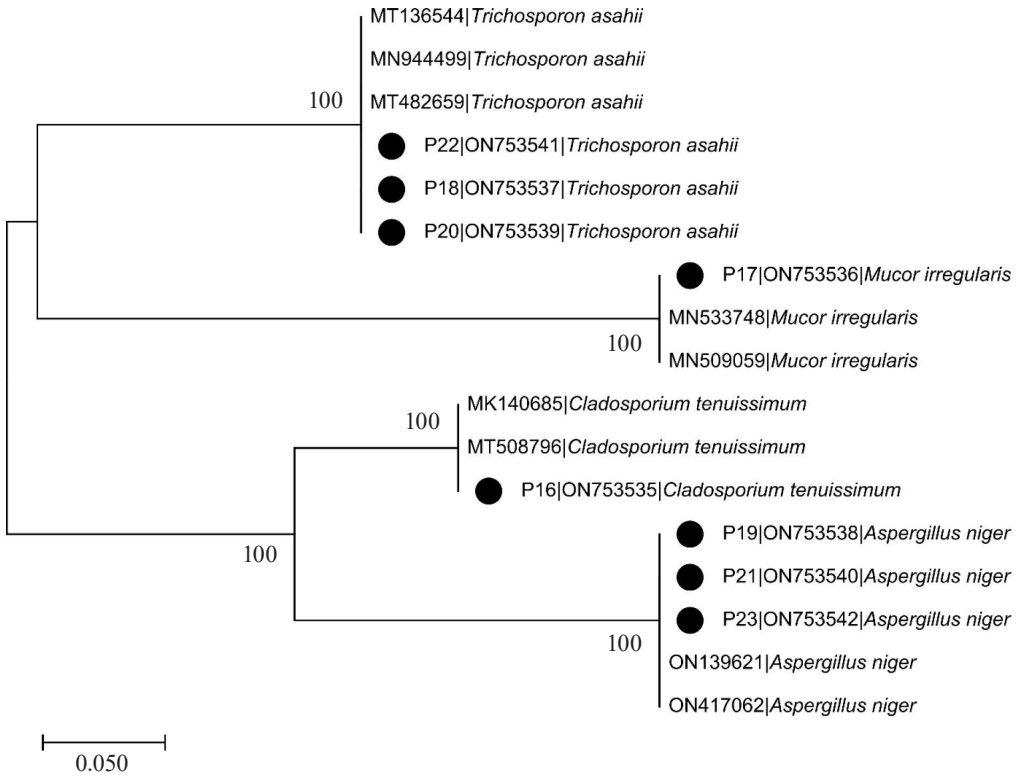
The DNA sequencing results using BLASTN identified the following fungi isolates (Fig. 4).

L. lactis and *L. fermentum* were the highest occurring bacteria isolates with percentage occurrences of 38.46 and 15.39%, respectively, while *L. fermentum* occurred least with a 7.69% occurrence (Fig. 5). Lactic acid bacteria are a phylogenetically heterogeneous group of Gram-positive bacteria that share metabolic and physiological characteristics [32]. These bacteria ferment carbohydrates into lactic acid (homofermentative), or into lactic acid, ethanol, and CO_2 (heterofermentative) [33, 34].

L. lactis is widely used in industry as a fast acidifier, starter culture, and flavor enhancer in milk fermentation [35–37]. In addition, *L. lactis* subsp. *lactis* is widely applied in cheese and butter production, as well as to inhibit pathogen development [38–41].

Food-associated *Lactobacillus* strains are "generally recognized as safe" (GRAS) microorganisms that have a major role in fermented milk production and in sourdough technology. In particular, some members of these strains called non-starter lactic cultures play a considerable role in developing cheese and dough aroma, texture, and flavor through ripening. They are currently being used in fortifying health-enhancing functional foods [42–44].

L. fermentum is a Gram-positive, non-spore-forming, rod-shaped probiotic bacterium belonging to the heterofermentative lactic acid bacterium. It is capable of fermenting carbohydrates and producing lactic and organic acids [45]. The bacterium has anti-diabetic probiotic



Note: The nodes without circular annotation represent highly similar species that were obtained as top hits from the NCBI (National Centre for Biotechnology Information)

Figure 4 A phylogenetic tree showing the classification and evolutionary relationship of the fungal isolates

Table 1 Morphological characteristics of the identified bacterial isolates

Bacteria	Shape	Color	Elevation	Surface	Margin	Transparency	Consistency	Diameter
Lysinibacillus macrolides	Gram-positive rod	Creamy	Raised	Smooth	Entire	Clear	Dry	Small
Klebsiella pneumoniae	Gram-negative rod	Greyish white to pink	Dome-shaped	Mucoid	Entire	Opaque	Dry	Medium
Lactococcus lactis	Gram-positive rod	Creamy	Raised	Smooth	Undulate	Clear	Dry	Small
Providencia stuartii	Gram-negative rod	Dull grey	Raised	Smooth	Entire	Clear	Dry	Large
Enterobacter cloacae	Gram-negative rod	Reddish	Flat	Smooth	Entire	Clear	Dry	Large
Limosilactobacillus fermentum	Gram-positive rod	Creamy	Raised	Smooth	Entire	Clear	Dry	Medium
Lactobacillus fermentum	Gram-positive rod	Creamy	Raised	Smooth	Entire	Clear	Dry	Medium
Staphylococcus edaphicus	Gram-positive cocci	Yellow	Raised	Smooth	Entire	Clear	Moist	Small
Bacillus flexus	Gram-variable rod	Creamy	Flat	Smooth	Entire	Clear	Dry	Small

Table 2 Actual names of the bacterial isolates and the summary of BLASTN similarities

Sample code	Accession number	BLASTN identity of sample	Percentage identity, %	Actual name of organisms
P1	ON747236	*Lactococcus lactis* MT 597556 MT 573759	100	*Lactococcus lactis*
P2	ON747237	*Limosilactobacillus fermentum* ON 495412	100	*Limosilaclobacillus fermentum*
P3	ON747238	*Lactobacillus fermentum* MN 907811	100	*Lactobacillus fermentum*
P4	ON747239	*Bacillus flexus* MT 645459 MT 585375	100	*Bacillus flexus*
P5	ON747240	*Lactococcus lactis* MT 573759 MT 597556	100	*Lactococcus lactis*
P6	ON747241	*Enterobacter cloacae* MT 613381 MN 826532	99	*Enterobacter cloacae*
P7	ON747242	*Providencia stuartii* MU 584987	100	*Prodidencia stuartii*
P8	ON747243	*Lactococcus lactis* MT 573759 MT 597556	100	*Lactococcus lactis*
P9	ON747244	*Lactococcus lactis* MT 573759 MT 597556	100	*Lactococcus lactis*
P10	ON747245	*Staphylococcus edaphicus* MT 604700 MT 585436	100	*Staphylococcus edaphicus*
P11	ON747246	*Lactococcus lactis* MT 573759 MT 597556	100	*Lactococcus lactis*
P12	ON747247	*Klebsiella pneumonia* ON 514273	100	*Klebsiella pneumonia*
P14	ON747248	*Lysinibacillus marcrolides* MK 942418 MT 218365	100	*Lysinibacillus marcrolides*
P15	ON747249	*Lactobacillus fermentum* MK 893985	100	*Lactobacillus fermentum*

Table 3 Morphological characteristics of the identified fungal isolates

Fungi	Shape	Color	Mycelia	Surface	Margin	Transparency	Consistency	Diameter
Trichosporon asahii	Irregular	Light beige/white	Raised	Smooth	Wide	Waxy	Dry with irregular folds	Large
Mucor irregularis	Irregular/ ellipsoidal	Whitish to slightly yellowish	Aerial mycelia	Wool-like	Entire	Opaque	Cottony	Small
Cladosporium tenuissimum	Rough walled	Olive green to brown/ black	Mycellium with swellings	Smooth	Undulate	Clear	Verruculose	Small
Aspergillus niger	Plethora strains	White to yellow/black	Conidial heads are radiate	Cottony	Entire	Opaque	Dry	Large

Table 4 Actual names of the fungal isolates and the summary of BLASTN similarities

Sample code	Accession number	Identity of sample	Percentage identity, %	Actual name of organisms
P16	ON753535	*Cladosporium tenuissimum* MK 140685 MT 508726	100	*Cladosporium tenuissimum*
P17	ON753536	*Mucor irregularis* MN 533748 MN 509059	100	*Mucor irregularis*
P18	ON753537	*Trichosporon asahii* MT 136544 MN 944499 MT 486259	100	*Trichosporon asahii*
P19	ON753538	*Aspergillus niger* ON 139621 ON 417062	100	*Aspergillus niger*
P20	ON753539	*Trichosporon asahii* MT 136544 MN 944499 MT 486259	100	*Trichosporon asahii*
P21	ON753540	*Aspergillus niger* ON 139621 ON 417062	100	*Aspergillus niger*
P22	ON753541	*Trichosporon asahii* MT 136544 MN 944499 MT 486259	100	*Trichosporon asahii*
P23	ON753542	*Aspergillus niger* ON 139621 ON 417062	100	*Aspergillus niger*

features which enable host cells to adjust their anti-inflammatory and antioxidant systems, resulting in improved glucose homeostasis capable of oxidative stress protection in diabetic conditions [46, 47]. It can be added to fermented foods like yoghurt and is found in some dietary supplements [48, 49]. In addition, *L. fermentum* was reported as the most predominant bacteria in Chinese cereal gruel, West African cereal dough, and Indian rice-based fermented beverage [50–52]. Furthermore, it is regarded as "generally recognized as safe" (GRAS) by the United States Food and Drug Administration (FDA) [53]. *L. fermentum* can also inhibit the growth of foodborne pathogens in food products [54]. In addition, foods obtained from fermentation by *L. fermentum* usually possess good palatability, high sensory quality, texture, stability, and nutritional properties [55, 56].

The spore-forming bacteria include *L. macroides* and *Bacillus flexus* with an occurrence of 7.69% (Fig. 5). They demonstrate an exceptional ability to adapt to their environment [55-56], and their presence in fermented flours could be due to their capacity to resist the acid produced by lactic acid bacteria [57–59]. Also, they are commonly found in beans and legumes [60]. *Bacillus* spp. present in fermented foods hydrolyze the substrate and produce enzymes, such as nattokinase, phytase, amylase, protease, cellulase, and lipase. These enzymes help break down complex compounds into simple biomolecules [61]. For example, amylase converts starch in legumes into sugar. Likewise, protease is used to convert proteins into amino acids [61, 62].

Bacillus species have also shown probiotic potential [63]. *B. flexus* biofilm has been used as a biologi-

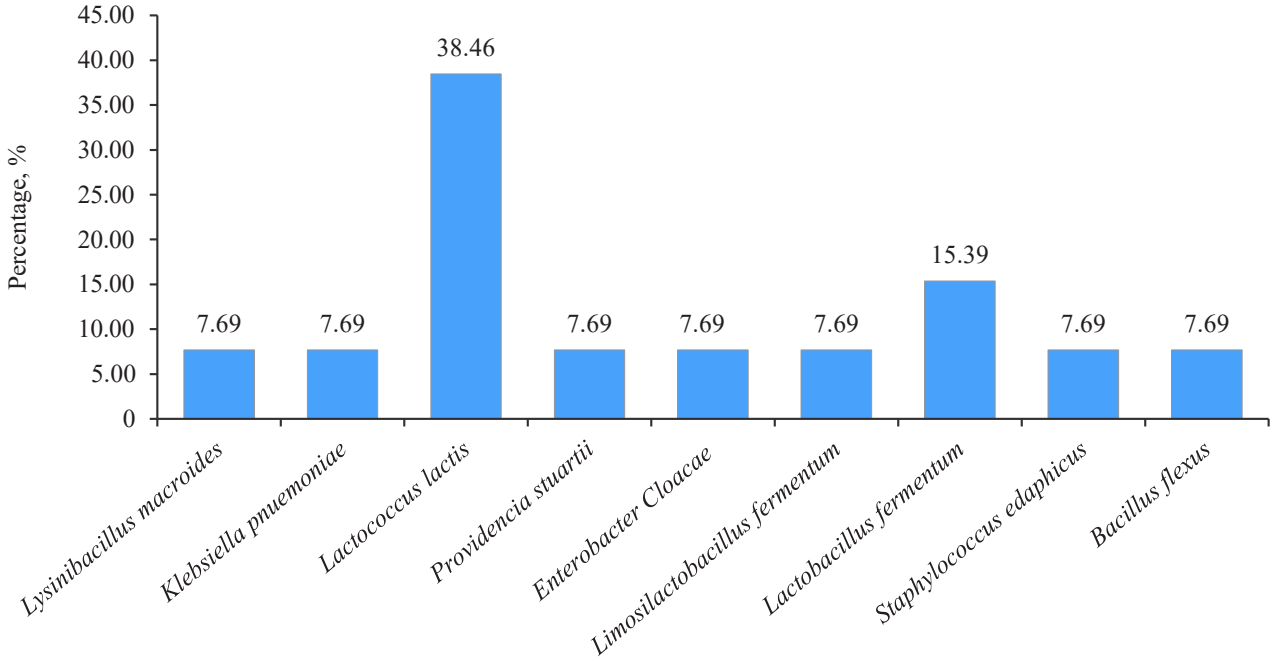


Figure 5 Percentage genera of the bacteria isolated from fermented botanicals

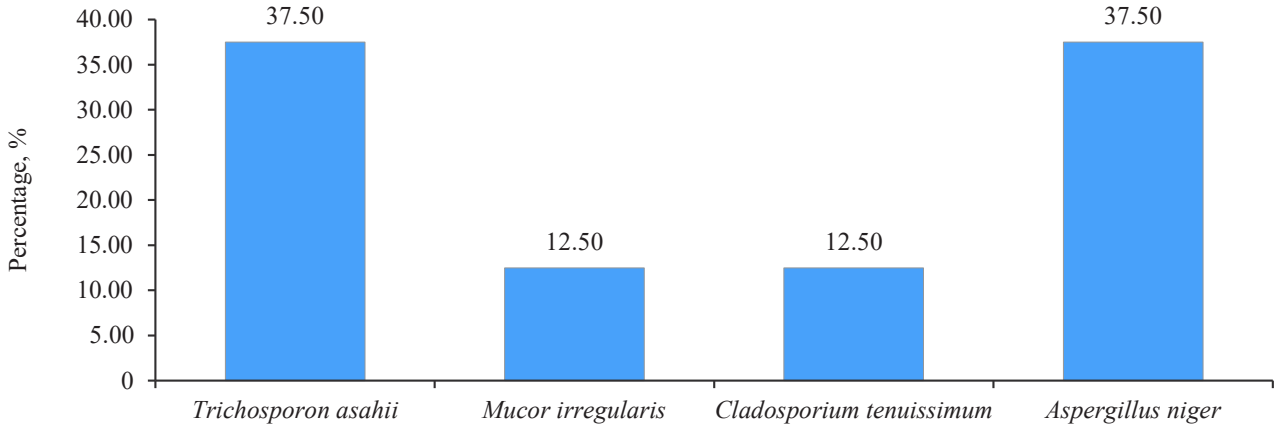


Figure 6 Percentage genera of the fungi isolated from fermented botanicals

cal control agent against the Cowpea pest *Callosobruchus maculates* [64].

The microbial profile of the fermented flour also showed the presence of *Enterobacteriaceae*. These include *Klebsiella pnuemoniae*, *P. stuarti*, and *E. cloacae* with a 7.69% occurrence each (Fig. 5). The *Enterobacteriaceae* family comprises a large group of Gram-negative non-spore-forming bacteria. These facultative anaerobic rods, which break down glucose-producing acid with/without gas, include some harmless commensal species, as well as important human and animal pathogens [65]. Their ubiquitous distribution means some members of the *Enterobacteriaceae* family will inevitably enter the food chain. While their low numbers are acceptable and do not directly lead to safety apprehension, their presence signifies inappropriate or poor processing and sanitary protocols around the food processing surroundings [66, 67]. Generally, *Enterobacteriaceae* are considered hygiene indicator organisms during food processing. Therefore, they are used to monitor the effectiveness of implemented preventive pre-requisite measures such as Good Manufacturing Practices and Good Hygiene Practices (GMP/GHP) [68].

According to percentage occurrence data for the fungi from fermented botanicals, both *T. asahii* and *A. niger* had a 37.50% occurrence, while *M. irregularis* and *Cladosporium temuissimum* had an occurrence of 12.50% (Fig. 6). These organisms are commonly present as contaminants in the human skin, cooking utensils, processing equipment, the environment, water, or in the seeds of cereals and legumes [69, 70]. They do not appear to play a significant role in the fermentation process, although they could be further exploited for their probiotic potentials [70–72].

CONCLUSION

The amalgamation of morphological attributes and molecular (DNA) markers have been accurately used for microbial nomenclature at the molecular level because the processes eliciting genetic changeability are the direct product of sequence changes from biochemical markers (genes and proteins). The phylogenetic properties of microbial isolates obtained from the fermentation of botanicals can provide valuable information on the diversity, composition, and functional properties of these microbial communities. Identification and characterization of the microorganisms involved in the fermentation process may optimize fermentation conditions to enhance the quality and nutritional value of the final products.

CONTRIBUTION

All the authors participated in developing the research concept and writing the original draft.

CONFLICT OF INTEREST

The authors have no conflict of interest concerning the conceptualization, research design, and publication of this work.

DATA AVAILABILITY

All the data associated with this study has been deposited in the NCBI GenBank database, with the accession numbers of ON747236-ON747249 (bacteria) and ON753535-ON753542 (fungi).

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