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IDENTIFICATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM SPONTANEOUSLY FERMENTED AFRICAN NIGHTSHADE (*SOLANUM SCABRUM*) LEAVES

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ABSTRACT

African nightshade (*Solanum scabrum)* is one of the most commonly consumed indigenous leafy vegetables in most parts of Sub-Saharan Africa. The vegetable is an excellent source of beta-carotene, vitamin A, C, E, iron, iodine, zinc, potassium, and protein, making it one of the most essential foods in addressing the malnutrition challenge among people experiencing poverty. This study focused on isolating, characterizing, and identifying dominant lactic acid bacteria (LAB) in naturally fermenting African nightshade leaves. The identification of LAB involved morphological, phenotypic, and molecular methods, including 16S rRNA gene sequencing. A total of 24 LAB strains were isolated and identified through phenotypic and 16S rRNA gene analyses. African nightshade fermentation was dominated by five genera of lactic acid bacteria: *Lactiplantibacillus plantarum* (38%), *Leuconostoc* spp (33%), *Levilactobacillus brevis* (13%), *Weissella* spp (13%) and *Lactococcus lactis* (3%). The findings reveal a rich and diverse community of LAB in fermented African nightshade, underscoring its potential as a natural reservoir of beneficial microorganisms. These LAB strains could have significant commercial and industrial potential, especially as starter cultures and probiotics in the production of fermented foods.

Keywords: Lactic acid bacteria, African nightshade, Spontaneous fermentation

INTRODUCTION

Africa is gifted with a wide variety of indigenous leafy vegetables (ILV), which are rich in vitamins and minerals. They are, thus, a valuable source of nutrition, especially in rural areas, where they can help alleviate micronutrient deficiency among the poor population **(Oguntoyinbo *et al*., 2016**). African nightshade (*Solanum scabrum),* a member of the *Solanaceae* family, is among the most consumed leafy vegetables in most parts of Sub-Saharan Africa.It is the largest species in its group, characterised by broad leaves. It is cultivated widely in many tropical African regions and in parts of East, South, and South East Asia, the South Pacific, North America, and the Caribbean **(Msewu & Mabuza, 2022**). African nightshade exhibits considerable diversity in terms of growth habits, leaf colour, and bitterness levels **(Abukutsa-onyango, 2015; Shackleton *et al*., 2009**)
Fermentation is a widely used preservation technique by millions of people in developing countries due to its affordability and accessibility to consumers (**Obafemi *et al*., 2022**). Fermentation involves the microbial transformation of carbohydrates, primarily facilitated by probiotic microorganisms. While fermentation is mainly known for breaking down sugars, it also affects other food components, changing both the flavour and functionality (**Mulaw *et al*., 2019**). Fruits and vegetables have served as good media for the growth of lactic acid bacteria (LAB) because of the high contents of vitamins, minerals, dietary fiber, and antioxidant compounds (**Wu *et al*., 2020**). Fermented food and beverage products have become increasingly known not only as a source of nutrients but also for their functional and probiotic properties, including protection against foodborne illnesses. Fermented vegetables have been shown to harbour a variety of microorganisms, with LAB being notable for producing lactic acid and sometimes bacteriocins that collectively extend shelf life and enhance food safety (**Mokoena *et al*., 2021**). The potential of lactic acid bacteria derived from fermented vegetables as probiotics has attracted significant interest in recent years **(Liu *et al*., 2022**). The microbial communities involved in vegetable fermentation are highly diverse and can significantly influence the safety and quality of the end product. The microbes primarily responsible for fermentation originate from the raw materials, but factors like brine composition, added ingredients, and the processing environment also influence microbial diversity(**Bautista-Gallego *et al*., 2020**).
LAB are a diverse group of Gram-positive microorganisms, typically aerotolerant and acid-resistant, and usually appear as non-spore-forming rods or cocci (**Ngasotter *et al*., 2020**). They are crucial in food fermentation, where they prevent the growth of spoilage and pathogenic microbes (**Ngasotter *et al*., 2020**).LAB can be categorized as heterofermentative, producing lactic acid along with other compounds like carbon dioxide, hydrogen peroxide, ethanol, and acetic acid (**Soundharrajan *et al*., 2021**).These metabolic by-products enhance food's nutritional and sensory qualities and contribute to its preservation and safety (**Oguntoyinbo *et al*., 2016**).
The initial steps of vegetable fermentations are usually carried out by heterofermentative microorganisms, which contribute to the final product's flavour and aroma by producing lactic and acetic acids (**Bautista-Gallego *et al*., 2020**). Consequently, as fermentation progresses, these microorganisms are succeeded by more acid-tolerant homofermentative species, whose ability to produce lactic acid leads to a substantial reduction in pH, thereby further suppressing the growth of undesirable microbial populations (**Kim *et al*., 2021**).
Indigenous leafy vegetables are crucial for the food security of smallholder farmers in rural and peri-urban areas of Kenya **(Gido *et al*., 2017**). However, inadequate production conditions can lead to post-harvest losses as high as 50%(**Onyango, 2016**). While the fermentation of vegetables, especially cabbage, has a long-standing tradition in Europe, the practice of fermenting indigenous leafy vegetables remains relatively uncommon in many parts of Africa. This presents an opportunity for intensifying efforts to discover and advance fermentation as a cost-effective preservation method for leafy vegetables across Africa (**Oguntoyinbo *et al*., 2016**). In this study, spontaneous fermentation of African nightshade leaves was carried out to isolate and characterise the LAB diversity during the fermentation process.

MATERIAL AND METHODS

Vegetable sourcing and sample preparation

Fresh African nightshade (*Solanum scabrum*) leaves were sourced from the research farms of Jomo Kenyatta University of Agriculture and Technology (JKUAT). The vegetables were manually harvested and transported to the laboratory in sterile plastic bags under hygienic conditions to minimise contamination. Upon arrival, the leaves were destemmed, thoroughly rinsed with tap water to remove debris and surface contaminants, and gently air-dried using a salad spinner.

Fermentation of African nightshade leaves

Spontaneous fermentation was carried out in a 10-litre stainless steel fermentation container. For each batch, 1kg of prepared nightshade leaves was submerged in 3 litres of brine solution containing 3% (w/v) table salt and 3% (w/v) commercial sugar. The brine was sterilised by autoclaving at 121ºC for 20 min to eliminate any pre-existing microbial population. The fermentation was conducted in duplicate and maintained at a constant temperature of 25ºC for 144 hours.

Microbial enumeration and pH determination

To monitor the fermentation process, samples were collected at predetermined times: 0, 24, 48, 72, 96, and 144 hours. Five (5)ml of fermentation brine was aseptically collected at each interval into sterile 15ml Falcon tubes for pH measurement using a calibrated pH meter. Simultaneously, 5ml of the brine was serially diluted tenfold in Ringer's solution for microbial analysis. From each dilution, 100 µl was plated on de Man Rogosa and Sharpe (MRS) agar and incubated at 30ºC for 24-48 hours. Bacterial colony-forming units (CFUs) were recorded to monitor changes in the LAB population throughout the fermentation process.

Isolation of presumptive lactic acid bacteria from fermented African nightshade leaves

De Man, Rogosa, and Sharpe (MRS) agar and broth used in isolation were purchased at Himedia, Mumbai, India. Liquid samples (1ml) were taken at 0, 24, 48, 72, 96, and 144 h, added to 9 ml quarter-strength Ringer's solution, and vortexed. The samples underwent serial 10-fold dilutions, and 100 µl aliquots were plated onto de Man, Rogosa, and Sharpe (MRS) agar. The plates were incubated at 30 °C for 24–48 hours. Colonies were randomly picked from the highest dilution plates for subsequent characterization. Selected isolates were cultivated aerobically in MRS broth at 30 °C and subsequently streaked to obtain pure cultures. Stock cultures of the isolates were stored in MRS broth containing 20 % glycerol at −80 °C.

Phenotypic Characterization of LAB isolates

Morphological characterization of presumptive LAB was based on classical macroscopic techniques of colour, form, shape, and elevation of pure colonies. Presumptive lactic acid bacteria were phenotypically characterised by determination of morphology by phase-contrast microscopy at 100× magnification (Shimadzu CX41, Japan), as well as standard tests such as catalase activity (**Cappuccino & Sherman, 2014**), gas production from glucose in MRS broth, growth at NaCl concentrations 6.5 %, growth at 10 and 45 ºC and at pH 2 and pH 4. Based on these phenotypic traits, the strains were categorized into three groups: obligately heterofermentative rods, facultatively heterofermentative and obligately homofermentative rods, and obligately heterofermentative cocci (**Wafula *et al*., 2023**).

Genotypic characterization of LAB isolates

Genomic DNA from all strains was extracted from overnight cultures grown in MRS broth at 30°C using the Quick-DNATM Fungal/Bacterial Miniprep Kit (Zymo Research, USA), following the manufacturer's instructions. The concentration and purity of the extracted DNA were assessed using a PCRmax Lambda spectrophotometer, while DNA integrity was evaluated through electrophoresis on a 1% agarose gel. PCR amplification of the 16S rRNA gene for presumptive LAB strains was done using bacterial universal primers 27 F:5`-AGA GTT TGA TCC TGG CTC AG-3` and 1492 R:5` GGT TAC CTT GTT ACG ACT T-3`. PCR was performed in a 50µl reaction containing 25µl One Taq® 2X Master Mix with standard buffer (New England Biolabs), 1µl forward primer, 1µl reverse primer, and 22 µl RNase-free water. Afterwards, 49 µl of the prepared reaction mixture was transferred into a sterile PCR tube, followed by adding 1 µl of genomic DNA, which served as the template. PCR amplification of the gene fragment was performed under the following conditions**:** an initial denaturation at 94 °C for 4 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 52 °C for 1 minute and 30 seconds, and extension at 68 °C for 1 minute. The reactions were carried out in a thermal cycler (ProFlex PCR systems). To verify the size of the 16S rRNA gene PCR products, samples were subjected to electrophoresis on a 1% (w/v) agarose gel stained with GelRed. The DNA bands were visualized using a gel documentation system (Uvitec Cambridge, UK). Following electrophoresis, PCR amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) according to the manufacturer's instructions. Purified DNA amplicons were sequenced via the Sanger method at Macrogen (Macrogen Europe B.V., Amsterdam, Netherlands).

Phylogenetic Analysis

The 16S rRNA gene sequences of the bacterial isolates were viewed for quality checks and edited using ChromasPro 2.2.0 software package (https://technelysium.com.au/wp/chromaspro/). They were then compared with available standard sequences of bacterial lineages in the public nucleotide sequence databases in the National Centre for Biotechnology Information (NCBI) using nucleotide blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find closely related bacterial 16S rRNA gene sequences. The 16S rRNA gene sequences of the isolates and those of the unknown closely related bacteria strains were aligned using Clustal W software, and phylogenetic trees were constructed using Kimura 2-parameter model with MEGA (Molecular Evolutionary Genetics analysis) 7.0 software package **(Kumar *et al*., 2016**). The trees' topologies were evaluated using the bootstrap resampling method (**Felsenstein, 1985**) based on 1000 replicates. The sequence of *Escherichia coli* was used as a control.

RESULTS AND DISCUSSION

Results

Microbial enumeration and pH determination

The microbial population peaked between 24 and 48 hours of fermentation, coinciding with the exponential growth phase of the microorganisms. A decline in microbial counts was observed between 72 and 144 hours, suggesting the transition into the stationary or death phase. The pH measurements throughout the fermentation process highlighted the acidifying capacity of LAB. A sharp decline in pH was noted from the initial value of 6.45 to 4.29 within the first 24 hours, followed by a gradual decrease to 3.98 at 48 hours and reaching approximately 3.51 by the end of the fermentation period. LAB counts, measured on MRS agar, started at log 2 CFU/ml and increased significantly to log 7 CFU/ml within 24 hours. These counts remained relatively stable, ranging from log 7 to log 8 CFU/ml, for the remainder of the fermentation, indicating LAB dominance on these selective media (Figure 1).

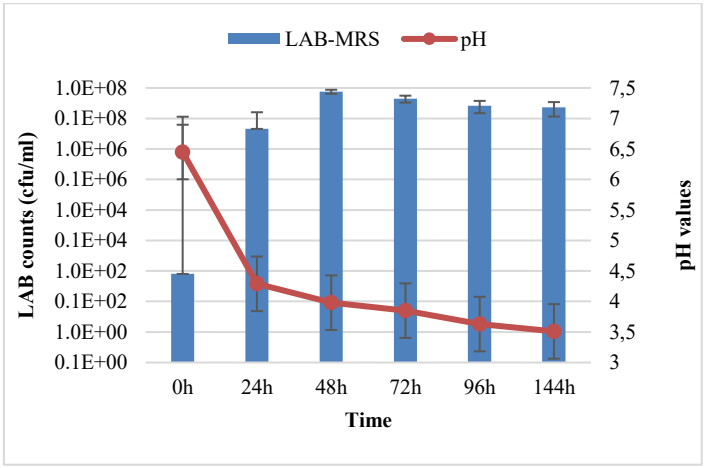

Figure 1 Average LAB counts (CFU/ml) in the fermentation brine of vegetable African Nightshade (VN), assessed using MRS. The graph also displays average pH values. Data represent mean values from duplicate fermentation experiments.

Phenotypic characterization

In the isolation of presumptive LAB, most colonies were able to grow within 1-2 days of incubation at 30ºC. The bacterial isolates were characterized through Gram staining, cell morphology, and catalase testing. All isolates appeared Gram-positive and catalase-negative, consistent with typical LAB characteristics. A total of twenty four presumptive lactic acid bacteria were isolated from the fermentation brine using MRS agar, as presented in Table 1. Among these, eight displayed a coccus morphology, while sixteen were rod-shaped, as presented in Table 1.

Most of the isolates grew in 6.5 % NaCl and at pH 4 (Table 1). Among the twenty four isolates, fourteen exhibited gas production during glucose fermentation. It was therefore classified as heterofermentative, while the remaining ten did not produce gas and were identified as homofermentative, in line with the criteria described by (**Kostinek *et al*., 2008**). Of the heterofermentative group, eight isolates were coccus-shaped and demonstrated gas production from glucose metabolism, indicating they likely belong to heterofermentative cocci, presumably within the genera *Leuconostoc* or *Weissella*. Additionally, six rod-shaped isolates also produced gas from glucose and were therefore identified as heterofermentative rods, potentially affiliated with the genera *Lactobacillus* or *Weissella*.

Table 1 Phenotypic Characterization and Molecular Identification of LAB Isolates

	Phenotypic Characterization									Molecular Identification		
					Growth at							
Sample ID	**cell shape**	**Gram**	**Catalase**	**CO_2**	**6.5% Nacl**	**10ºC**	**45ºC**	**pH 2**	**pH 4**	**Closest relatives**	**% identity**	**Accession no.**
NSR246	Rod	+	-	+	+	-	-	-	-	*Weissella cibaria*	99.64	MT464147.1
NSR481	Cocci	+	-	+	+	-	-	-	-	*Leuconostoc mesenteroides*	98.68	MT544985.1
NSR482	Cocci	+	-	-	-	-	-	-	-	*Lactococcus lactis*	98.81	MT645510.1
NSR485	Cocci	+	-	+	+	-	-	-	-	*Leuconostoc mesenteroides*	99.13	MT545098.1
NSR488	Cocci	+	-	+	+	-	-	-	-	*Leuconostoc citreum*	99.73	MT544678.1
NSR721	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.45	0M980098.1
NSR729	Rod	+	-	+	+	-	-	-	+	*Weissella cibaria*	99.69	MT613505.1
NSR7211	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.67	MT515842.1
NSR7216	Cocci	+	-	+	-	-	-	-	-	*Leuconostoc mesenteroides*	99.13	MT545098.1
NSR7217	Cocci	+	-	+	-	-	-	-	-	*Leuconostoc mesenteroides*	99.13	MT545098.1
NSR7218	Cocci	+	-	+	-	-	-	-	-	*Leuconostoc pseudomesenteroides*	99.7	MT544883.1
NSR7219	Cocci	+	-	+	-	-	-	-	-	*Leuconostoc pseudomesenteroides*	99.34	MT544883.1
NSR961	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.67	MT515842.1
NSR964	Rod	+	-	+	+	-	-	-	+	*Levilactobacillus brevis*	99.61	MT544691.1
NSR1441	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.67	MT515842.1
NSR1443	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.67	MT515842.1
NSR1444	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.45	MT611923.1
NSR726	Rod	+	-	+	-	-	-	-	-	*Levilactobacillus brevis*	99.92	MT640328.1
NSR248	Rod	+	-	+	+	-	+	-	+	*Weissella confusa*	99.23	MT613537.1
NSR1449	Rod	+	-	+	+	-	-	-	+	*Levilactobacillus brevis*	99.87	MT495904.1
NSR1442	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.76	MT613640.1
NSR1445	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.76	MT613640.1
NSR1446	Rod	+	-	-	+	-	-	-	+	*Lactiplantibacillus plantarum*	99.76	MT613640.1
NSR966	Cocci	+	-	+	-	-	-	-	-	*Leuconostoc mesenteroides*	98.48	MT545098.1

Genotypic characterization of LAB isolates

Based on the 16S rRNA gene sequencing results in the GenBank database, the eight heterofermentative strains that were coccus-shaped and produced gas from glucose fermentation thus belonged to the genus Leuconostoc and accounted for 33% (*Leuconostoc mesenteroides, Leuconostoc pseudomesenteroides,* and *Leuconostoc citreum* with the sequence similarity ranging from 98-99% (Table 1). The six strains that were rod-shaped produced gas from glucose metabolism and were identified with 16S rRNA gene sequencing as *Levilactobacillus brevis* (13%) with sequence similarity of 99 %, and 13% strains belonged to the genus *Weissella* with sequence similarities of 99% (*Weissella cibaria* and *W. confusa)* (Table 1).

Among the 10 homofermentative isolates, nine were identified as Gram-positive, rod-shaped, catalase-negative, and showed no gas production from glucose fermentation, characteristics consistent with homofermentative rods. Based on 16S rRNA gene sequencing, these isolates exhibited 99% sequence similarity to *Lactiplantibacillus plantarum* (Table 1).

In addition, one isolate was Gram-positive, coccus-shaped, catalase-negative, and did not produce gas during glucose fermentation. This strain could grow in 6.5% NaCl but failed to grow at either 10 °C or 45 °C, as well as under acidic conditions at pH 2 or 4. The 16S rRNA gene sequencing identified these isolates as *Lactococcus lactis*, with 98% similarity (Table 1)

Phylogenetic analysis of the isolates

Phylogenetically, LAB isolated from African nightshade leaves had five distinct clusters corresponding to genera *Lactiplantibacillus, Weissella, Leuconostoc, Lactococcus,* and *Levilactobacillus* (Figure 2). Within the phylogenetic cluster corresponding to the genus *Lactiplantibacillus*, strains NSR721, NSR7211, NSR961, NSR1441, NSR1443, NSR1444, NSR1442, NSR1445, and NSR1446 grouped closely with *Lactiplantibacillus plantarum*, showing high sequence similarity to reference strains MT613640.1, MT515842.1, and MT611923.1.

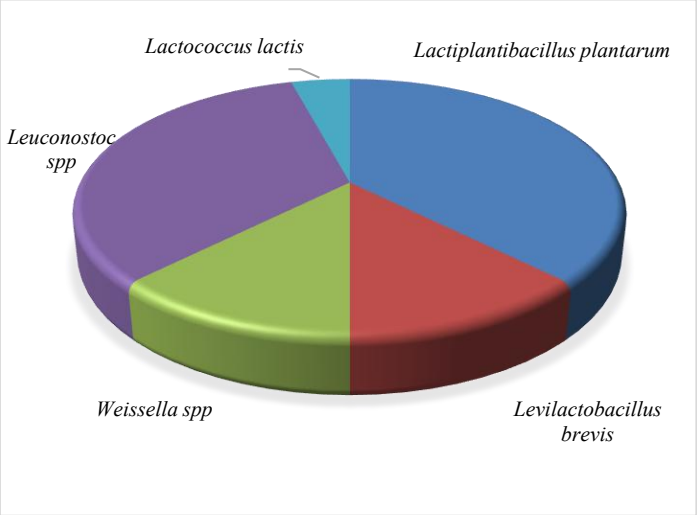

Figure 2 LAB isolated from African nightshade leaves grouped at the species level

Strains NSR726, NSR964, and NSR1449 were associated with *Levilactobacillus* brevis (MT640328.1, KM495904.1 and MT544691.1). Strains NSR246, NSR248 and NSR729 were associated to *Weissella cibaria and W.confusa* (MT613537.1 and MT464147.1) while strains NSR488, NSR966, NSR7219, NSR7216, NSR481, NSR7217, NSR485 and NSR7218 formed a cluster associated to *Leuconostoc sp*., while strain NSR482 was associated with *Lactococcus lactis* (MT645510.1) (Figure 3).

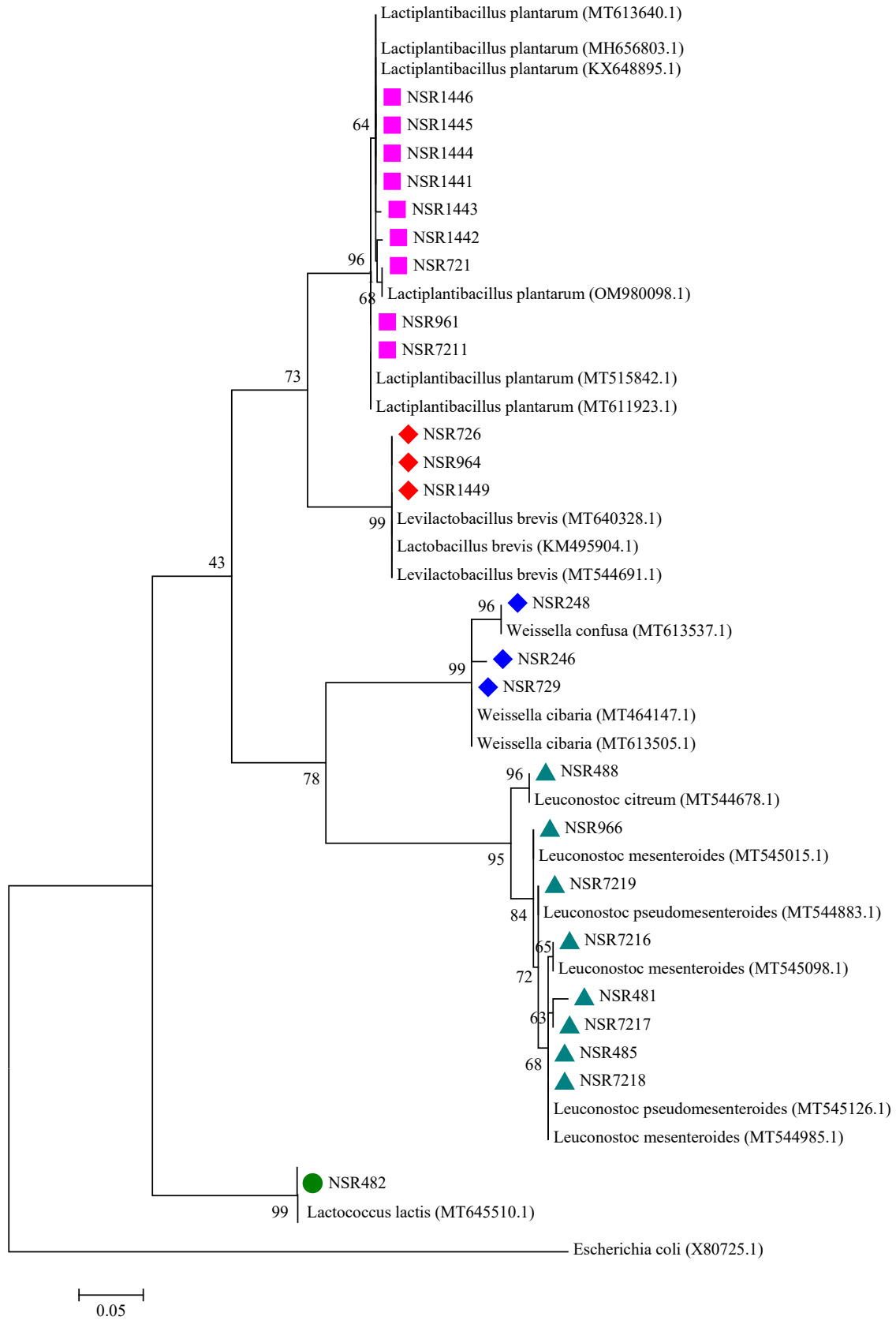

Figure 3 Phylogenetic tree based on 16S rRNA gene sequences showing the relationships of lactic acid bacterial isolates with related taxa. Scale bar = 0.10 substitutions per site. Bootstrap values are indicated at nodes; GenBank accession numbers are shown after strain names.

DISCUSSION

This study explored the spontaneous fermentation of African nightshade, an indigenous leafy vegetable commonly consumed in Kenya. African nightshade was selected due to its rich micronutrient profile and accessibility, as well as its affordability, making it a valuable dietary component for local populations. African indigenous vegetables often contain higher levels of vitamins and minerals compared to many exotic species (**Gido *et al.*, 2017**). Fermentation is an important cost-effective biotechnological process associated with increased product shelf life, enhanced nutritional value, improved sensory qualities, enhanced food product safety, improved palatability and digestibility (**Oguntoyinbo *et al.*, 2016**).

The primary objective was to characterize the LAB population throughout fermentation and examine the diversity of LAB species using phenotypic and biochemical profiling, further confirmed through 16S rRNA gene sequencing Fermentation was facilitated using a 3% sugar and 3% salt brine, designed to

mimic traditional fermentation practices while favoring LAB proliferation. The significant increase in LAB counts from approximately log 2 CFU/ml at 0 hours to log 7 CFU/ml at 24 hours demonstrates a robust microbial response, indicating the substrate's suitability for LAB growth. This rapid growth phase likely reflects the dominance of fast-acidifying, heterofermentative LAB, which can thrive in mildly acidic and osmotic environments. The subsequent stabilization of LAB counts (log 8 CFU/ml) through 144 hours, alongside a consistent drop in pH to below 4.0, signifies not only microbial stability but also successful inhibition of spoilage and pathogenic organisms (**Oguntoyinbo, *et al*., 2016a**), making the fermentation microbiologically safe.

Importantly, pH reduction is a central feature of successful vegetable fermentations, as it both preserves the food and contributes to flavor and texture. The sharp pH drop observed (from 6.45 to 4.29 within 24 hours and to 3.51 at 144 hours) aligns with earlier studies on vegetable fermentation (**Snyder *et al*., 2020**), confirming that LAB metabolism plays a critical role in acidification and microbial succession.

Molecular identification using 16S rRNA gene sequencing revealed a diverse LAB population, including *Lactiplantibacillus*, *Weissella*, *Leuconostoc*, *Lactococcus*, and *Levilactobacillus* (**McFeeters *et al*., 2013; Wafula *et al*., 2016**) .This diversity highlights the complexity of microbial dynamics in spontaneous fermentation, where no external starter cultures are introduced. Such diversity is beneficial, as different LAB species contribute unique metabolic products that enhance flavor, texture, and safety. The presence of these genera aligns with prior studies on plant-based fermentations and reinforces the importance of substrate type and native microflora in determining fermentation outcomes (**Oguntoyinbo, *et al*.,2016a**)

Phylogenetic analysis confirmed that the dominant isolates were *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Weissella cibaria*, *Weissella confusa*, and *Leuconostoc* spp., reflecting the diverse LAB community present during spontaneous fermentation. Notably, nine isolates (NSR721, NSR7211, NSR961, NSR1441, NSR1442, NSR1443, NSR1444, NSR1445, and NSR1446) were affiliated with *Lactiplantibacillus plantarum*, underscoring its ecological fitness and dominance, particularly in the late stages of fermentation. This species is biologically significant due to its acid and salt tolerance, as well as its versatile metabolic capabilities including the degradation of plant phenolics and production of antimicrobial peptides traits that contribute to both its survival and enhancement of the sensory and safety qualities of fermented foods.Further,eight isolates (NSR481, NSR485, NSR488, NSR7216, NSR7217, NSR7218, NSR7219, and NSR966) were linked to *Leuconostoc* spp., while three (NSR726, NSR964, and NSR1449) aligned with *Levilactobacillus brevis*, and three (NSR246, NSR248, and NSR729) were identified as *Weissella* spp. These findings indicate the prominent roles these genera play throughout the fermentation process. Similar LAB diversity has been reported in plant-based fermentations by (**Yilmaz *et al*., 2022**), in traditional foods such as kimchi and sauerkraut where *Lactiplantibacillus plantarum* and *Leuconostoc* species are frequently dominant (**Garcia-Gonzalez *et al*., 2021**).

Six isolates identified between 24 and 48 hours NSR246, NSR481, NSR482, NSR2485, NSR488, and NSR248 were classified under the genera *Weissella*, *Lactococcus*, and *Leuconostoc*, indicating their early involvement in the spontaneous fermentation of *Solanum scabrum*. These genera, commonly associated with the initial stages of vegetable fermentation, are typically heterofermentative and contribute to rapid acidification, gas production, and flavor development (**Behera *et al*., 2018**).Their early detection in this study aligns with the concept of microbial succession, where initial colonizers create conditions such as a lowered pH that enable more acid-tolerant species to thrive. This ecological transition is not merely a passive shift but reflects dynamic microbial interactions, with early LAB paving the way for successor strains. As fermentation progressed and the pH dropped below 4.0, more acid-resilient genera such as *Lactiplantibacillus plantarum* became dominant. At 144 hours, when acidity was highest, isolates NSR1441, NSR1442, NSR1443, NSR1444, NSR1445, and NSR1446 (Table 1) were all affiliated with *L. plantarum*, highlighting its adaptive advantage in low-pH environments. These findings reinforce previously reported microbial succession trends in spontaneous vegetable fermentations (**Li *et al*., 2023**).

Three isolates of *Weissella cibaria* were identified, a species described by (**Kang *et al*., 2016**) as a Gram-positive, non-motile, heterofermentative LAB unable to produce dextran from sucrose. Both *W. cibaria* and *W. confusa* are known to synthesize exopolysaccharides (EPS), such as dextran, which offer potential functional applications in the food industry, particularly as prebiotic fibers or hydrocolloid replacements in baking (**Arendt *et al*., 2015**).

Leuconostoc species also play significant roles in fermentation. For instance, *Leuconostoc mesenteroides* is a starter culture for its flavor-enhancing properties in dairy, vegetable, and coffee fermentations. *Leuconostoc lactis*, known for its EPS production, may also have valuable applications in food technology (**Saravanan & Shetty, 2016; Kot *et al*., 2014**). Some LAB genera identified in this study, particularly *Lactobacillus* and *Weissella*, have also been reported in earlier studies investigating fermented kimchi using 16S rRNA gene sequencing**(Gebru & Sbhatu, 2020**). Other authors, (**Paramithiotis, 2025**), have noted the consistent presence and dominance of *Lactiplantibacillus plantarum* in fermented plant materials. In the present study, *Lactiplantibacillus plantarum* accounted for 38% of the total isolates, demonstrating its resilience and adaptability in fermentation. Its ability to thrive in high salt and low pH conditions makes it a key contributor to the stability of fermented vegetables such as cucumbers, sauerkraut, and olives (**Behera *et al*., 2018**).

CONCLUSION

This study provides important microbiological and molecular insights into the spontaneous fermentation of African nightshade (*Solanum scabrum*), successfully isolating and characterizing a diverse community of lactic acid bacteria (LAB) through a combination of phenotypic methods and 16S rRNA gene sequencing. The identification of multiple LAB genera, including *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Weissella cibaria*, *Weissella confusa*, and *Leuconostoc* spp., highlights the microbial complexity of this traditional fermentation process. Notably, *L. plantarum* was the most frequently isolated species, underscoring its ecological dominance and potential functional relevance. These findings suggest that LAB strains from fermented African nightshade hold promise for application as starter cultures, probiotics, or natural food preservatives, contributing to the development of functional and shelf-stable foods. To build on these outcomes, future studies should investigate the probiotic potential, antimicrobial activity, and fermentative performance of these isolates, while leveraging advanced molecular and metagenomic tools to explore microbial dynamics under varying fermentation conditions, processing techniques, plant varieties, and geographic regions.

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