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Phenotypic and technological properties of *Leuconostoc mesenteroides* O157:H7 isolated from soybean cake (*okara*)

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Abstract

Lactic acid bacteria (LAB) are microorganisms that produce lactic acid as a by-product during metabolic activities. This work aimed to phenotypically and technologically characterize the *Leuconostoc mesenteroides* strain, among other presumptive LAB strains isolated from soybean cake (*okara*). Okara is a solid waste resulting from sieving during the production of soymilk. The isolate was subjected to morphological, biochemical, and technological tests during characterization. The morphological and biochemical tests revealed that the isolate was presumptively, a lactic acid bacterium, *Leuconostoc mesenteroides*. Technological properties of the isolate showed capability for starch hydrolysis, indicating the potential of the isolate to improve flavour and texture of fermented foods if applied as a starter culture. Although the isolate could not hydrolyse arginine, it showed a positive indication for lipase activity which may also contribute to flavour development in fermented foods. Furthermore, the isolate showed negative haemolytic activity hence, non-virulent. The isolate was assessed for its ability to aggregate with pathogens; *Vibrio parahemolyticus*, *Staphylococcus aureus*, and Escherichia coli O157: H7 and possibly inhibit them. The result ranged from 57.42% (Staphylococcus aureus) to 78.62% (*Escherichia coli* 0157). The isolate also showed Autoaggregation of 57.20 % which means that it also has a strong aggregation ability.

Keywords: Okara; Phenotypic analysis; Technological properties; Lactic acid bacteria

1. Introduction

Okara is a byproduct generated during the industrial production of soy milk and tofu, comprising the insoluble fractions of soybeans that remain after the filtration of pureed soybeans. Despite its nutrient-rich composition, okara is underutilised and is frequently discarded, contributing to environmental pollution. However, due to its biochemical composition, okara presents a promising substrate for the cultivation and proliferation of microorganisms, including lactic acid bacteria (1).

Lactic acid bacteria are important microorganisms that produce lactic acid as a by-product during metabolic activities. The food industry always seeks strains with superior characteristics and properties to enhance sensory and product quality. This highlights how important lactic acid bacteria are in the food industry. During fermentation, lactic acid bacteria produce organic acids and other metabolites that enhance flavour development in food, and prevent spoilage, and are thus very useful in many applications, especially in the food and dairy industry Lactic acid bacteria also possess therapeutic properties that are vital for human health enhancement. According to Olaoye et al. (2), lactic acid bacteria are regarded as generally safe (GRAS) and have been widely used as starters in the production of fermented foods.

Lactic acid bacteria (LAB) are Gram-positive, catalase-negative, non-sporulating, microaerophilic microorganisms that constitute a natural component of the human gut microbiota. The primary genera within LAB include *Lactococcus,*

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Lactobacillus, Leuconostoc, Pediococcus, Enterococcus, and *Streptococcus* (3). Based on their metabolic pathways, LAB can be classified as homofermentative, wherein lactic acid constitutes at least 85% of the fermentation products, or heterofermentative, characterized by the production of additional metabolites such as acetate, ethanol, and carbon dioxide. Homofermentative lactic acid bacteria (LAB) are frequently incorporated into starter cultures due to their ability to facilitate rapid acidification in food fermentation processes. Conversely, heterofermentative LAB are primarily utilized as components of non-starter cultures, contributing to the enhancement of sensory attributes through their glycolytic, lipolytic, and proteolytic activities, as well as the production of diacetyl (4). Additionally, numerous LAB strains are classified as probiotics, indicating their potential to confer health benefits to the host when consumed in sufficient quantities. Emerging research suggests that LAB play a role in modulating immune responses, cancer prevention, and the management of airway inflammation, with efficacy in addressing gastrointestinal disorders (5). This study aimed to isolate and assess the phenotypic and technological properties of a lactic acid bacteria from okara.

2. Material and methods

2.1. Materials and sample collection

Soybean was purchased from a main market in Ebonyi state, Nigeria. The soybean was processed into soymilk and the waste product after sieving (*okara* or cake) was aseptically taken to the laboratory for isolation of the presumptive lactic acid bacteria.

2.2. Study Area

The research was carried out at Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

2.3. Morphological and Biochemical Analysis

- ***Isolation of Leuconostoc mesenteroides O157:H7 from soymilk cake (okara)***: This was done using a modified method described by Olaoye (6). Ten grams (10 g) of naturally fermented soymilk cake (*okara*) was mixed with 90 ml of sterile normal saline. A series of appropriate dilutions of the sample was done with the same diluent. by measuring 1 ml of the diluent into 9 ml of Normal saline to make 10^{-1} dilution. The tube was mixed well, and 1 ml of the mixture was pipetted into the second tube (10^{-2}), and mixed. The process was repeated sequentially to prepare up to (10^{-5}) for other tubes. One (1) ml of each dilution was introduced into sterile plates in triplicate. Subsequently, 20 ml of sterilised De Man, Rogosa and Sharpe (MRS- Oxoid) agar was poured into the plates, gently rocked, and allowed to solidify. The inoculated plates were incubated at 37°C for 48 h in an anaerobic Jar (Biolab Scientific- Canadian) with a CO_2 gas kit. Plates with 20-300 colonies were enumerated and the results expressed as $\log_{10}$ of colony-forming units per millilitre (log CFU/ml) of the samples.
- ***Gram staining reaction:*** A modified method of Cheesbrough (7) was used. Overnight cultures of bacterium isolate on nutrient agar were smeared on the surface of clean glass slides to form a thin layer, and the slide was left to air dry before it was heat fixed. The slide was first flooded with crystal violet solution (FIRMTEC BIOSCIENCES NIGERIA) for 60 seconds and rinsed under running water, then fixed with Lugol's iodine (LOBA CHEMIE INDIA) for 60 seconds and rinsed. Aceto-alcohol (50% acetone and 50% ethanol) (FIRMTEC BIOSCIENCES NIGERIA) was used to decolourise the slide. Lastly, the slide was counter-stained with safranin solution (FIRMTEC BIOSCIENCES NIGERIA) and allowed to stand for another 60 seconds then washed off with running water. The slide was observed under the light microscope (LOBA CHEMIE INDIA) using ×100 objective lens (oil immersion).
- ***Catalase test***: This test was carried out according to the method previously described by Cheesbrough (7). Catalase test was carried out by putting a drop of 3% (v/v) hydrogen peroxide (FIRMTEC BIOSCIENCES NIGERIA) solution on a clean grease-free slide. A sterile wire loop was used to pick the test organism and mix it with the hydrogen peroxide. The formation of bubbles indicated a positive reaction.
- ***Coagulase test***: This was done using the procedure described by Cheesbrough (7). A drop of sterile distilled water was placed on each end of a sterile slide. Then a colony of the test organism was emulsified on each spot to make two tick suspensions. A loopful of plasma was added to one of the suspensions and mixed gently. The slide was examined for clumping or clotting of the organisms within 10 seconds. Plasma was not added to the second suspension which served as control. Clotting indicated a positive result while no clotting indicated a negative result.
- ***Spore staining***: The malachite green-Schaeffer and Fulton Method, Prescott *et al.,* (8) was adopted in endospore staining. A thin smear of culture on a glass slide was placed on a staining rack above boiling water. The smear was covered with small pieces of paper towel and kept saturated with malachite green (5% aqueous

solution) with heating for about 5 min, then washed gently with water. The slide was counter-stained with safranin for 30 seconds, washed with water and blot-dried. The slide was examined under the oil immersion objective (9).

- ***Motility test***: This test was done by hanging drop technique as described by Cheesbrough (7) A drop of normal saline was placed on a sterile slide and the colony of the test organism was suspended and emulsified, then covered with a cover slip. The slide was examined microscopically using a 10x and 40x objective lens. Movement in different directions gave a positive result.
- ***Oxidase test***: This test was done according to the method of Cheesebrough (7). The oxidase reagent used was 1% solution of tetramethyl-p-phenylenediamine dihydrochloride (Sigma Aldrich). The filter paper was soaked with few drops of freshly prepared oxidase reagent. A colony of the test organism was smeared on the filter paper using a sterilized stick. The development of blue - purple within ten (10) seconds was recorded as positive result and no colour was recorded as negative result.
- ***Citrate utilisation test***: The isolates were streaked on Simmons citrate agar (Titan Biotech Ltd, India) slant and incubated at 37°C for 24 hours and the colour change was observed. Isolates that utilised citrate as an energy source were indicated by deep blue colour formation within 24-48 hours (7).
- ***Urea utilisation test***: The method of Brink (10) was adopted with slight modification. Christensen's Urea Agar (Titan Biotech Ltd, India) was prepared and sterilised at 121°C. Urea solution was added to make 10 % (V/V) solution, then dispensed into test tubes and allowed to solidify in slant form. Heavy inoculum from 24-hour pure culture was streaked on the entire slant surface. The butt was not stabbed as it served as a colour control. All the tubes were incubated at 37°C with loosened caps. The culture slant was observed for a colour change at 6 hours, 24 hours, and 48 hours. Urease production was indicated by a bright pink colour on the slant that extends into the butt.
- ***Carbohydrate fermentation test***: Different sugar media were prepared by dissolving 2 % Mannitol, Sucrose, Glucose, Xylose, and Lactose in different solutions containing 0.5 % yeast extract, Durham's tube and supplemented with andrade's indicator. Bacteria cultures 24 hours old were inoculated into the tubes and were incubated at 37°C for 24 hours. Acid production (the ability of the test organism to ferment each sugar) was indicated by a change in colour of the media to pink. The gas formation was observed in Durham's tubes (11).
- ***Growth of bacterial isolate at different temperatures***: Overnight LAB cultures (1% v/v) were inoculated into MRS broth and incubated at different temperatures of 20°C, 25°C, 30°C, 35°C and 40°C respectively for 24 h. Their growths were determined by measuring their turbidity using the spectrophotometer at 600 nm, and subsequently seeded on MRS agar plates and incubated for 24 – 48 h at 37 °C. The appearance of LAB colonies on MRS agar plates corresponded with and confirmed their growth in MRS broth (12).

2.4. Screening of Isolate for Probiotic Properties

- ***Ethanol tolerance***: For the ethanol tolerance, 5%, 10%, 15%, 20% and 25% concentration of Ethanol containing nutrient broth (Hitech media - India) was prepared and sterilized in different test tubes. The organism was inoculated into the test tube containing the ethanol in their different concentrations and incubated at 24 h at 37 °C. This test was carried out on the isolate to test its ability to survive in an alcoholic environment. Growth was measured by recording turbidity at 600 nm with UV Spectrophotometer (13).
- ***Acid tolerance***: For pH tolerance, the organism was inoculated into the test tube containing ten millilitres of sterile Nutrient Broth with different pH concentrations of 2, 3, 4, 5, 6, and 7 were inoculated with 100 μl overnight grown culture and incubated at 37 °C for 48 hours. Growth was measured by recording turbidity at 600 nm with a UV Spectrophotometer (14).
- ***Salt tolerance (NaCl)***: This test was carried out on the probiotic isolate to test its ability to survive in high concentrations of NaCl. Five microliters of cultures were inoculated into sterile 5 ml Nutrient broth with different concentrations of NaCl viz; 5%, 10%, 15%, 20% and 25% and incubated at 37°C for 24-48 hours and growth was measured at 600 nm using UV Spectrophotometer (12).
- ***Bile salt tolerance***: Cultures (24 h) were streaked on the nutrient agar medium containing different bile salt concentrations 0.5%, 1.0%, 1.5%, 2.0% and 2.5%. The plates were incubated at 37°C for 48 hours, after incubation the extent of growth was recorded objectively based on visible growth on the plate and marked as triple positive sign (+++) for maximum growth, double positive sign (++) for moderate growth, single positive sign (+) for poor growth and negative sign (-) for no growth (12).
- ***Phenol tolerance of the isolate***: MRS broth (5 ml) was modified with 0.1%, 0.2%, 0.3%, 0.4% and 0.5% phenol (PubChem) to determine the phenol tolerance of the isolate. One (1) per cent of the overnight bacterial culture was inoculated to the culture medium. Un-inoculated phenol medium served as negative control. Growth was observed based on turbidity at 600 nm using a UV Spectrophotometer (12).
- **Antibiotic Resistance of the Isolate:** A modified method based on CLSI (15) guidelines was adopted to assess the antibiotic susceptibility of bacterial strains. The antibiotic disc diffusion method was used to evaluate the

isolates' resistance to various antibiotics. The tested antibiotics and their concentrations were as follows: Ceftriaxone (10 µg), Ampicillin (10 µg), Cloxacillin (10 µg), Ciprofloxacin (10 µg), Gentamycin (10 µg), Chloramphenicol (10 µg), Vancomycin (30 µg), and Clindamycin (30 µg). Bacterial cultures were prepared in MRS broth and adjusted to a 0.5 McFarland turbidity standard. The standardized inoculum was spread onto Mueller-Hinton agar plates (Hitech Media, India). Antibiotic discs were then placed on MRS agar plates, and the plates were incubated at 37°C for 48 hours in an anaerobic jar with a gas kit. After incubation, the diameter of the inhibition zones around each antibiotic disc was measured in millimetres (mm) using a transparent ruler. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (15) and categorized as either sensitive or resistant.

- ***Determination of the antimicrobial activity of the isolate***: The agar well diffusion method was employed to assess the antimicrobial activity of the isolates. The antimicrobial properties of the isolate were tested against major food-borne pathogenic bacteria namely *Staphylococcus aureus, Escherichia coli, Bacillus subtilis and P. aeruginosa* using the modified method described by CLSI (15). The selected isolate was inoculated from slants to fresh 250 ml MRS broth and incubated at 37°C for 48h. The culture broth of the isolate was centrifuged separately at 10,000 rpm for 30 m. The supernatant was collected and passed through a 0.2 µm sterile syringe filter. The cell-free neutral supernatant broth was collected for the antibacterial study against selected food-borne pathogens. A 24-hour culture of the pathogens was suspended in normal saline and standardised by comparing with 0.5 MacFarland solution. A lawn of cells was made by spreading the cell suspension over the surface of MRS agar plates with a sterile swab stick. The plate was allowed to dry, and a sterile cock borer of diameter (5 mm) was used to create uniform wells in the agar. Each well was filled with 200 µl culture-free supernatant and then incubated at 37°C for 24 hours. The plate was observed for a zone of inhibition (ZOI) around the well. Results were considered positive if the diameter (mm) of the ZOI was greater than 10 mm, and negative if less than 10 mm.
- ***Bacterial adhesion to hydrocarbons (hydrophobicity assay)***: The hydrophobicity of the bacterial strain was performed by xylene extraction according to Reuben *et al.* (12) with little adjustment. The isolate was cultivated in MRS broth at 37°Cfor 20 hours. The bacterial cells were harvested by centrifugation at 10,000 × g for 10 min at 4°Cand washed twice with phosphate-buffered saline (PBS; pH 7.2), and then re-suspended in the PBS (pH 7.2). The absorbance at 600 nm (OD600) was adjusted to 0.25 ± 0.05 to standardize the number of bacteria (approximately 10^8 CFU/mL). Then an equal volume of xylene was added. The 2-phase system was thoroughly mixed by a vortex for 4 min. The aqueous phase was removed after 1 h of incubation at room temperature and its absorbance at 600 nm was measured. The affinity of the bacterial strains to hydrocarbons (hydrophobicity) was reported as adhesion percentage.
- ***Autoaggregation Assay***: Autoaggregation assays were carried out according to Reuben *et al.* (12) with some modifications. Lactic acid bacteria isolate was cultivated in MRS broth at 37°Cfor 20 hours. The bacterial cells were harvested by centrifugation at 10,000 × g for 10 min at 4°Cand washed twice with phosphate-buffered saline (PBS; pH 7.2), and then re-suspended in the PBS (pH 7.2). The absorbance at 600 nm (OD600) was adjusted to 0.25 ± 0.05 to standardise the number of bacteria (approximately 10^8 CFU/mL). The absorbance at 600 nm of cell suspensions (4 mL) incubated at 37°Cwas monitored with a spectrophotometer for 5 h at different times (0 and 5 h).
- ***Coaggregation assay***: A coaggregation assay was carried out according to Reuben *et al*. (12) with some modifications. Exactly 2 mL of each pathogen bacterial suspension (*Escherichia coli* O157: H7, *Vibrio* spp., *Staphylococcus aureus*) were mixed and incubated at 37°Cwithout agitation. The absorbance of the mixtures described above was monitored at different times (0 and 5 h). Absorbance was determined for the bacterial suspensions alone. The coaggregation percentage was calculated.
- ***Starch hydrolysis test***: The isolate was streaked on a starch agar plate and incubated at 37°C for 24 hours. After incubation, the plates were flooded with lugol's iodine solution (LOBA CHEME) and observed for a transparent zone surrounding the colony with no blue-black formation (14).
- ***Arginine hydrolysis***: Overnight grown culture was inoculated into Arginine broth medium (Sigma Aldrich), and the tubes were incubated at 37°C for 24 hours, after incubation 100 µl of culture was transferred onto a white background and the same amount of Nessler's reagent (Sigma Aldrich) was pipette on the culture. The appearance of a bright orange colour indicates a positive reaction, while the yellow colour indicates a negative reaction. Arginine-free broth was used as a control (14).
- ***Lipase activity***: Overnight-grown culture of isolate was spotted on Tributyrin agar (Liofilchem) and incubated at 37°C for 72 hours. The opaque zone around the colonies indicated lipase activity. Cellulase Enzyme Active culture of isolate was streaked on carboxymethyl Cellulose agar (Sigma Aldrich) and incubated at 37°C for 48 hours. The plates were stained with 0.1 % Congo red stain (LOBA CHEME) followed by washing with 1 M NaCl. The positive result indicated the formation of a clear zone of hydrolysis (16).

- ***Hemolytic activity***: Bacterial isolate was spot inoculated on Blood agar (supplemented with 5% sheep blood-Hitech Media). The plates were incubated for 48 hours at 30℃. A clear zone around the colony was observed and considered a positive result (12).

3. Results and Discussion

3.1. Cultural, morphological, and biochemical characteristics of *Leuconostoc mesenteroides* O157:H7

Table 1 provides the cultural and morphological characteristics of *Leuconostoc mesenteroides* O157:H7 isolated from soybean cake (*Okara*). The preliminary tests like grams staining, endospore staining, catalase test, motility test and coagulase test made it possible to classify the isolate as potential probiotic lactic acid bacteria. The microscopic observation and biochemical reaction revealed that the isolate was a lactic acid bacteria strain and was gram-positive, cocci, catalase-negative and non-sporing. The isolate was also non-motile. According to a study by O'Flaherty *et al.* (17), some Gram-positive bacteria are good probiotics due to their ability to withstand the acidic environment of the gastrointestinal tract. Catalase activity can be a distinguishing feature, as many lactic acid bacteria (LAB) are catalase-negative. The presence of catalase does not necessarily disqualify a strain from being a probiotic but can influence its behaviour in the gut. LAB are coagulase-negative and generally regarded as safe. This is very typical of the isolate, *Leu. Mesenteroides* in the present investigation as coagulase-positive bacteria such as *Staphylococcus aureus* can be pathogenic. The isolate was also non-sporulating. The result of this investigation is corroborated by (18,13).

Table 1 Cultural and morphological characteristics of *Leuconostoc mesenteroides* O157:H7

Cultural characteristics	Features
Morphology	Yellow, irregular, small
Shape	Cocci
Gram reaction	+
Catalase	-
Coagulase	-
Spore staining	No spore
Motility	Non- motile

+ = Positive; - = Negative.

Table 2 Phenotypic characteristics of *Leuconostoc mesenteroides* O157:H7

Phenotypic characteristics	Reaction
Oxidase	-
Indole	-
Citrate	+
Urea	-
Maltose	+
Lactose	+
Fructose	+
Sucrose	+
Glucose	+
Xylose	+
Mannitol	-
Galactose	+

Key: **+** = Positive reaction**;** **-** = Negative reaction.

Table 2 shows the phenotypic characteristics of *Leuconostoc mesenteroides* O157:H7. The oxidase reaction of the isolate is in support of the biochemical characteristics of LAB which confirm them to be oxidase-negative. The inability of bacteria to convert tryptophan to indole indicates the strong tendency of probiotic bacteria as typical LAB like *Lactobacillus* and *Leuconostoc* which are indole-negative, as shown by the LAB isolate being reported. This result is similar to the findings of Olaoye *et al.,* (2), who reported that all lactic acid bacteria screened were non-indole producers. Similarly, the LAB isolate was citrate-positive, urea negative aligning with probiotic characteristics (19). The result of different sugar ultilisation assays by the LAB isolate indicated that the isolate fermented a wide range of sugars including maltose, lactose, fructose, sucrose, glucose, xylose, mannitol, and cellobiose, which is typical of *Leuconostoc* species, known for their diverse carbohydrate fermentation capabilities as reported (20).

3.2. Effect of Temperature on the Growth of *Leuconostoc mesenteroides* O157:H7

Table 3 shows the effect of temperature on the growth of the LAB isolate. These findings demonstrate typical growth patterns for LAB isolates under varying temperatures. The optimal growth for the lactic acid bacteria isolate was about 35°C. These findings are consistent with the literature on LAB growth and temperature preferences (Rahman et al. (21). This temperature is conducive to the fermentation processes of probiotic strains. A study by Rahman et al. (21) reported that LAB generally exhibits optimal growth at temperatures around 37°C. The growth of the isolate at higher temperatures (up to 40°C) indicates the strain may have a broader temperature tolerance, which could be advantageous in various fermentation conditions. This discovery can be supported by Rahman et al. (21), who observed that some LAB strains (Bifidobacterium spp.) can tolerate and grow well at higher temperatures (45°C). In the same vein, a decrease in growth at 40°C for the isolate suggests that while LAB. can tolerate slightly higher temperatures, extreme temperatures beyond their optimal range can negatively affect their growth, as noted by Corsetti and Rossi (22).

Table 3 Effect of temperature on the growth of *Leuconostoc mesenteroides* O157:H7

Temperature	Optical density
20°C	0.08b ± 0.03
25°C	0.7a ± 0.09
30°C	2.43a ± 0.65
35°C	2.72a ± 0.09
40°C	2.19 ab ± 0.49

Data are the mean of results from triplicate experiments ± the standard deviation. A higher value of optical density indicates more growth.

3.3. Effect of Ethanol, pH and NaCl on the Growth of *Leuconostoc mesenteroides* O157:H7

Table 4 shows the effect of different ethanol, pH and NaCl concentrations on the growth of the LAB isolate. Results showed that the isolate could optimally tolerate 5% - 20%, slightly 25% ethanol concentration. The LAB isolate showed a significant decline in growth with increasing ethanol concentrations. Ethanol tolerance is an important factor for applications of LAB in fermentation, most importantly where ethanol (by-product) accumulation is significant.

Acid tolerance is an important criterium for the selection of probiotic bacteria as they must be able to survive in the acidic gastric environment. To survive passage through the gastrointestinal tract, resistance to low pH is important. The LAB. isolate was positive to the acidic pH. This result is in line with the report of Odu et al. (13) who isolated acid-tolerant Lactobacillus species from traditionally fermented ogi, palmwine, and tigernut , and the isolated strain survived at pH 3. The isolate exhibited a clear preference for higher pH levels. This suggests that the isolate is less tolerant to acidic conditions and performs optimally in neutral to alkaline environments.

The effect of different concentrations of NaCl that inhibit the growth of certain bacteria was assessed on the LAB isolate. Studies show that bacterial strains with moderate halotolerance can adapt to a range of salinity conditions (23). The isolate shows significant growth at 5% NaCl but declines sharply at higher concentrations, with little or no detectable growth at 15%, 20%, and 25% NaCl. This suggests minimal tolerance to salinity. The salt resistance test results of some reports provided evidence of the sodium chloride tolerance nature of some of the Lactobacillus spp. (13,24). The result is consistent with existing literature on responses of lactic acid bacteria to saline conditions. The lack of growth at higher NaCl concentrations (15% and above) for the isolate reflects a common trend observed in microbial studies where

extreme salinity significantly hampers growth. Research suggests that high NaCl concentrations can disrupt cellular processes, leading to osmotic stress and growth inhibition (25).

Table 4 Effect of ethanol, pH and NaCl on the growth of *Leuconostoc mesenteroides* O157:H7

Ethanol		**pH**		**NaCl**	
Concentration (%)	**Optical density**	**Concentration**	**Optical density**	**Concentrations (%)**	**Optical density**
5	0.73 ± 0.01^{b}	pH 2	0.1 ± 0.03^{a}	5	0.65 ± 0.17^{d}
10	0.71 ± 0.07^{b}	pH 3	0.41 ± 0.14^{b}	10	0.19 ± 0.02^{c}
15	0.63 ± 0.11^{b}	pH 4	0.63 ± 0.05^{a}	15	0.08 ± 0.005^{c}
20	0.33 ± 0.07^{b}	pH 5	0.72 ± 0.07^{a}	20	0.00 ± 0.0^{c}
25	0.23 ± 0.02^{b}	pH 6	0.83 ± 0.16^{a}	25	0.00 ± 0.0^{b}
-	-	pH 7	0.85 ± 0.33^{b}		-

Data are the mean of results from triplicate experiments ± the standard deviation. A higher value of optical density indicates more growth.

3.4. Growth of *Leuconostoc mesenteroides* O157:H7 at Different Bile Salt and Phenol Concentrations.

The growth of *Leuconostoc mesenteroides* O157:H7 at different bile salt and phenol concentrations is shown in Table 5. Bile salt tolerance is among the crucial parameters for the selection of probiotics and they must be able to survive bile fluid secreted in the small intestine which destroys the bacterial cell membranes and affects cell permeability and viability of the bacteria. The ability of the bacterial isolates to tolerate bile was assessed by the growth of bacterial isolates at various concentrations of bile salts ranging from 0.5%- 2.5% for 48 hours. The isolate tolerated 0.5% to 1.0 % bile salt concentrations as it recorded maximum growth. At 1.5%, there was moderate growth while the isolate had a poor growth at 2.0% to 2.5 % concentration of bile salt. The results of the gastric juice resistance were similar to the results of Rahman *et al.* (21) who reported that *Lactobacillus* spp. was able to survive in a gastric juice environment at pH 2.2. The phenol resistance test results of this report provide evidence that the *Lactobacillus* isolate tolerated phenol at 0.1 and 0.2%, and 0.3% phenol. The isolate could not tolerate higher concentrations of the phenol concentration after 12, and 24 hours of incubation. Studies have demonstrated that phenol can be toxic to microorganisms, inhibiting growth and metabolic activities. Bacteria have shown variable tolerance to phenol, often linked to their ability to degrade or detoxify the compound.

Table 5 Growth of *Leuconostoc mesenteroides* O157:H7at different bile salt and phenol concentrations

Bile salt		**Phenol**	
Concentration (%)	**Result**	**Concentration (%)**	**Result**
0.5	+++	0.1	+
1.0	+++	0.2	+
1.5	++	0’3	+
2.0	+	0.4	-
2.5	+	0.5	-

Keys: (+++) for maximum growth, double positive sign (++) for moderate growth, single Positive sign (+) for poor growth and negative sign (-) for no growth. While + = positive result, - = negative result for phenol.

3.5. Antibiotics Susceptibility of *Leuconostoc mesenteroides* O157:H7

Table 6 shows the antibiotic susceptibility of the selected bacteria isolate. Lactic acid bacteria sensitivity to antibiotics is the most important factor in their safety evaluation. Antibiotic resistance is a potential risk of probiotic application, as horizontal transfer of the antibiotic resistance gene has been reported between *Lactobacilli* and *Enterococcus faecalis* (26). The results obtained in this study showed that there was multi-drug resistance by the isolate. There was high susceptibility of the probiotic isolates to Chloramphenicol and Ceftriaxone, whereas there was a high level of tolerance to Cloxacillin, Ciprofloxacin, Vancomycin, Clindamycin, Gentamycin, and Ampicillin. According to Klare *et al.* (27), LAB are usually sensitive to antibiotics such as Chloramphenicol. The *Lactobacillus* strain in the present study showed

tolerance for Vancomycin. This discovery is consistent with the report of Tulumoglu *et al.* (28) who found that 90% of the *Lactobacillus* strains tested were resistant to Gentamicin. Also, there is a high level of resistance of the LAB isolates to Ampicillin in the present study.

Table 6 Antibiotic susceptibility of *Leuconostoc mesenteroides* O157:H7

Antibiotics	**Zones of inhibition (mm) of Isolates**
Ceftriaxone (10 μg)	17(S)
Ampicillin (10 μg)	0 (R)
Cloxacillin (10 μg)	0 (R)
Chloramphenicol (10 μg)	16(S)
Ciprofloxacin (5 μg)	12(R)
Gentamicin (30 μg)	12 (R)
Vancomycin (30 μg),	0(R)
Clindamycin, (20 μg)	0(R)
MARI	0.75

Key: R- Resistant; S- susceptible. MARI- Multiple antibiotics resistant index

3.6. Antimicrobial Activity of *Leuconostoc mesenteroides* O157:H7

Table 7 gives the results for the antimicrobial activity of the LAB isolate in terms of the diameter of the zone of inhibition (ZOI). A diameter >10 mm around the well was considered as a positive result. The antimicrobial properties were tested against major food-borne pathogenic bacteria namely: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Pseudomonas aeruginosa*. The LAB isolate showed potent antimicrobial activity against the pathogenic organisms. Antimicrobial activity is an important property of potential probiotic bacteria in the inhibition of pathogenic organisms and preventive gastrointestinal infection (29). The inhibition by *Lactobacillus* spp*.,* against these pathogenic bacteria has been reported in a previous study (30). This report also indicated that Gram-positive bacteria are more sensitive to *Lactobacillus* than Gram-negative bacteria. The LAB strain could inhibit both Gram-positive and negative bacteria, thus showing a wide antimicrobial spectrum. The lowest inhibiting effect was observed on *Pseudomonas aeruginosa.* The inhibition of growth of the test pathogens by the *Lactobacillus* isolate suggests its antimicrobial activity and potential to stop the proliferation of pathogenic bacteria.

Table 7 Antimicrobial activity of *Leuconostoc mesenteroides* O157:H7

Antimicrobial Activity	**Zone of inhibition (mm) of LAB**
Staphylococcus aureus	10.0 ± 1.02
E. coli	6.0 ± 1.1
Bacillus subtilis	4.0 ± 1.0
Pseudomonas aeruginosa	1.0 ± 2.62

Data are the mean of results from triplicate experiments ± the standard deviation. Higher value of optical density indicates more growth.

3.7. Technological Properties of *Leuconostoc mesenteroides* O157:H7

The technological properties of LAB isolate are shown in Table 8. The isolate showed capability for starch hydrolysis activity. This means that the isolates can improve the texture and flavour of fermented foods by breaking down complex carbohydrates. The isolate cannot hydrolyze arginine to help improve the flavour as it showed negative for arginine hydrolysis. There was a positive result for lipase activity which can enhance the flavour of dairy products by breaking down fats into free fatty acids. The isolate showed negative haemolytic activity, and this indicates the isolate was non-virulent nor pathogenic. The result of haemolytic activity test performed proved the safety of the selected LAB isolate,

as an absence of haemolytic activity is the primary condition for the selection of probiotic organisms (31). The results obtained in the present study were similar to observations by Gupta *et al.* (32) and Thakkar *et al.* (14).

Table 8 Technological properties of *Leuconostoc mesenteroides* O157:H7

Technological properties	Result
Starch Hydrolysis	+
Arginine Hydrolysis	-
Lipase Activity	+
Hemolytic Activity	-

Key: + positive result, - negative result

3.8. Aggregation and Hydrophobicity Abilities of *Leuconostoc mesenteroides* O157:H7

Coaggregation involves the assessment of the abilities of probiotic strains to aggregate with pathogens and possibly inhibit them. Table 9 shows the Aggregation and Hydrophobicity Abilities of the selected LAB isolate. Results of Coaggregation ranged from 57.42% (*Staphylococcus aureus)* to 78.62% (*Escherichia coli* 0157).

Autoaggregation assay measures the ability of probiotic strains to aggregate with themselves. When there is a higher percentage, it indicates a stronger aggregation. Cell hydrophobicity, a property of the cell surface, is crucial for assessing the adhesion capabilities of *Lactobacillus*. For lactic acid bacteria (LAB), this characteristic is a key factor in evaluating the suitability of a strain for use as a starter culture. The isolate showed a high hydrophobicity, indicating good adhesion capabilities.

Table 9 Aggregation and Hydrophobicity Abilities of *Leuconostoc mesenteroides* O157:H7

Aggregation and Hydrophobicity		Percentage
Coaggregation assay:	*Vibrio parahemolyticus*	58.10 ± 0.69
	Staphylococcus aureus	57.42 ± 3.7
	Escherichia coli O157: H7	78.62±5.71
Autoaggregation assay		57.20 ± 1.58
Hydrophobicity Assay		86.7 ± 0.8

Data are the mean of results from triplicate experiments ± the standard deviation. A higher value of optical density indicates more growth.

4. Conclusion

This work successfully isolated and phenotypically characterized *Leuconostoc mesenteroides O157:H7* from *Okara,* a solid waste from the production of soymilk. The isolate further demonstrated its potential for industrial and probiotic applications following its technological properties. These properties confirmed the identity of the isolate. The study has added to the growing knowledge of the indigenous lactic acid bacteria, suggesting its possible application in food fermentation processes with potential health benefits.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Aritonang, S. N., Roza, E., Rossi, E., Purwati, E., and Husmaini. (2017). Isolation and identification of lactic acid bacteria from okara and evaluation of their potential as candidate probiotics. Pakistan Journal of Nutrition, 16(8), 618-628.

[2] Olaoye, O. A., Ohuche, J. C., Nwachukwu, A. C., & Nwaigwe, U. V. (2022). Development of a starter culture for the improvement in the quality of ogiri, a food condiment. Official Journal of European High-tech and Emerging Research Association (EUHERA), 3(1), 37–44. https://doi.org/10.28991/HIJ-2022-02-01-01

[3] Motta, A. D. S., and Gomez, M.D. S. M. (2015). Technological and functional properties of lactic acid bacteria: The importance of these microorganisms for food. Revista Do Intstituto de Lacticinios Candido Tostes, 70. Article 172.

[4] Gurrasi, V., Sannino, C., Moschetti, M., Bonna, A., Di Grigoli, A., and Settanni, L. (2017). The individual contribution of starter and non-starter lactic acid bacteria to the volatile organic compound composition of Caciocavallo Paleermitano cheese. International Journal of Food Microbiology, 259, 35-42.

[5] Rama, G. R., Kuhn, D., Beux, S., Maciel, M. J., and Volken, de Souza, C. F. (2019). Potential applications of dairy whey for the production of lactic acid bacteria cultures. International Dairy Journal, 98, 25-37.

[6] Olaoye, O. (2014). Technological properties of Lactococcus lactis subsp. lactis I23 isolated from Nigerian beef and characterization of bacteriocin produced by it. British Biotechnology Journal, 4(7), 829–845. https://doi.org/10.9734/bbj/2014/11719

[7] Cheesbrough, M. (2006). District laboratory practice in tropical countries (Part 2, 2nd ed.).

[8] Prescott, L. M., Harley, J. P., & Klein, D. A. (2002). Laboratory exercises in microbiology. McGraw-Hill.

[9] Bisen, P. S. (2014). Microbial staining. In Microbes in Practice (pp. 139–155). New Delhi, India: IK International.

[10] Brink, B. (2010). Urease test protocol. American Society for Microbiology, 1–7.

[11] Stiles, M. E., & Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current taxonomy. International Journal of Food Microbiology, 36(1), 1–29. https://doi.org/10.1016/S0168-1605(96)01233-0

[12] Reuben, R. C., Roy, P. C., Sarkar, S. L., Alam, R. U., & Jahid, I. K. (2019). Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC Microbiology, 19, 1-20.

[13] Odu, N. N., Amadi, L. O., & Aguamah, E. O. (2021). Screening of bacterial isolates with probiotic potential of tiger nut drink, ogi, and palm wine. Journal of Applied Life Sciences International, 24(7), 36-48.

[14] Thakkar, P., Modi, H. A., & Prajapati, J. B. (2015). Isolation, characterization and safety assessment of lactic acid bacterial isolates from fermented food products. International Journal of Current and Applied Sciences, 4, 713-725.

[15] Clinical and Laboratory Standards Institute. (2020). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (11th ed.). CLSI guideline M07-A11.

[16] Taheri, H. R., Moravej, H., Tabandeh, F., Zaghari, M., & Shivazad, M. (2009). Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. Immunology, Health, and Disease, 1586-1593.

[17] O'Flaherty, S. J., & Klaenhammer, T. R. (2010). Functional and phenotypic characterization of a protein from Lactobacillus acidophilus involved in cell morphology, stress tolerance, and adherence to intestinal cells. Microbiology, 156, 3360–3367. https://doi.org/10.1099/mic.0.043158-0

[18] Rahman, M. M., Hossain, K. M., & Rahman, S. M. (2016). Isolation, characterization, and properties study of probiotic lactic acid bacteria of selected yoghurt from Bangladesh. African Journal of Microbiology Research, 10(1), 23-31.

[19] Nwachukwu, U., George-Okafor, U., Ozoani, U., & Ojiagu, N. (2019). Assessment of probiotic potentials of Lactobacillus plantarum CS and Micrococcus luteus CS from fermented milled corn-soybean waste-meal. Scientific African, 6, e00183.

[20] Sathyapriya, A., & Anitha, A. (2019). Assessment of probiotic potential *Leuconostoc mesenteroides* from natural microbiota. International Journal of Science and Research in Biological Science, 5(6), 26-33.

[21] Rahman, S. M. K., Das, B. K., Soikat, F. K., Rahman, S. M., & Hossain, K. M. (2015). Characterization of probiotic bacteria isolated from regional chicken feces. African Journal of Microbiology, 9, 880-885.

[22] Corsetti, A., & Rossi, J. (2002). Lactic acid bacteria in sourdough fermentation. Food Research International, 35(5), 239–248. https://doi.org/10.1016/S0963-9969(01)00105-3

[23] Qiu, G., Liu, J., & Zhao, Z. (2021). The effects of NaCl on microbial growth: A meta-analysis. Biotechnology Advances, 49, 107-118.

[24] Priyanka, G., Priyanka, P., Zalak, R., & Baldev, P. (2022). Isolation, characterisation, and identification of probiotic bacteria from fruits and vegetables. 12-23.

[25] Harris, M., Nguyen, T., & Lee, Y. (2019). Cellular responses to high salt concentrations in bacteria. BMC Microbiology, 19(1), 32.

[26] Ouoba, L. I. I., Lei, V., & Jensen, L. B. (2008). Resistance of potential probiotic lactic acid bacteria and bifidobacteria of African and European origin to antimicrobials: Determination and transferability of the resistance genes to other bacteria. International Journal of Food Microbiology, 121, 217-224.

[27] Klare, I., Konstabel, C., Werner, G., Huys, G., Vankerckhoven, V., Kahlmeter, G., Hildebrandt, B., Müller-Bertling, S., Witte, W., & Goossens, H. (2007). Antimicrobial susceptibilities of Lactobacillus, Pediococcus, and Lactococcus human isolates and cultures intended for probiotic or nutritional use. Journal of Antimicrobial Chemotherapy, 59, 900-912. https://doi.org/10.1093/jac/dkm032

[28] Tulumoglu, S., Yuksekdag, Z., Beyatli, Y., Simsek, O., Cinar, B., & Yaşar, E. (2013). Probiotic properties of lactobacilli species isolated from children's feces. Anaerobe, 24, 36–42.

[29] Kuti, K., Hussaini, I. M., Usman, A., & Isa, A. (2021). Isolation of Lactobacillus species from fermented Parkia biglobosa seed and screening for their probiotic activity. Agricultural Science and Technology, 13(2), 212–216.

[30] Asurmendi, P., García, M. J., Pascual, L., & Barberis, L. (2015). Biocontrol of Listeria monocytogenes by lactic acid bacteria isolated from brewer's grains used as feedstuff in Argentina. Journal of Stored Products Research, 61, 27–31.

[31] Padmavati, T., Rayavarapu, P. B. P., Rao, N. N., Ranganath, P. P., & Veerabadrappa. (2018). Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. Journal of Genetic Engineering and Biotechnology, 16(2), 357-362.

[32] Gupta, M., & Bajaj, B. K. (2018). Functional characterization of potential probiotic lactic acid bacteria isolated from Kalarei and development of probiotic journal of fermented oat flour. Probiotics and Antimicrobial Proteins, 10(4), 654–661.