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Microbial conversion of agro-wastes for lactic acid production

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ABSTRACT

Lactic acid (LA) is an organic compound produced *via* fermentation by microorganisms that can utilize a wide range of carbohydrate sources and has gained relevance in food industries for preservation, pharmaceutical industries as additives, textile industries as mordants and production of cosmetics and bioplastics. In spite of the various applications of lactic acid, its availability is a challenge, especially in developing countries like Nigeria and therefore has to be imported, making it very expensive. One of the core substrates for lactic acid production is glucose. This study focused on the production of lactic acid using reducing sugar from locally sourced agro-wastes (corncob, sugarcane bagasse, plantain peduncle and groundnut shell). Bacteria were isolated from the agro-wastes dumpsite and screened for lactic acid production using De Man Rogosa and Sharpe (MRS) agar. The agro-wastes were pretreated using NaOH and hydrolyzed using cellulase produced from *Aspergillus niger*. The lactic acid bacteria (LAB) were identified based on their cultural, morphological, biochemical and molecular characteristics. The screened isolates were used for the production of lactic acid in an MRS medium containing the agro-waste hydrolysates as the sole carbon source. The LAB isolate with the best ability for lactic acid production was ascertained using the spectrophotometric method. Eleven (11) isolates were obtained from the agro-wastes dumpsites. *Lactiplantibacillus plantarum* (accession number OM510300) from plantain peduncle dumpsites had the highest potential for lactic acid production (1.9558 g/L). The study revealed that Nigerian-locally sourced agro-wastes can be developed as an alternative source of reducing sugar for lactic acid production and thus leads to agro-wastes management in the environment.

Introduction

Lactic acid - LA (α-hydroxypropionic acid) is an abundant, hygroscopic, naturally occurring acid that easily dissolves in water [1]. It is the most basic hydrocarboxylic acid with an optically active chiral carbon atom. It is odourless and has a colour range from yellow to a colourless liquid state at 15 °C and 1 atm [1]. Lactic acid exists in two distinct enantiomeric forms, L (+) lactic acid and D (-) lactic acid (Ruiz Rodriguez et al., 2019, [2]). Lactic acid plays a crucial role in traditional food fermentations as well as in probiotics,

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potential medicinal products, and microbial factories for the production of organic antimicrobial agents that may be employed as preservatives in food products to enhance the nutritional quality and prolong the life span of beverages and food products [3]. Lactic acid has also gained importance in the cosmetics, chemical, and textile industries [4].

The abundance of agro-wastes is found littering the Nigerian environment. These wastes are gradually becoming a nuisance as they contribute greatly to environmental pollution including deterioration of land and pollution of several water bodies because they are not being efficiency utilized. Currently, no known agro-waste management policy exists in Nigeria. Agro-waste still remains as litter or pollutants in the environment. The generation of these wastes is associated with post-harvest operations or after processing of crop products [5,6]

Toxic compounds are released into the environment from the burning of agro-wastes including but not limited to particle pollution, volatile organic compounds, carbon monoxide, nitrous oxide, and other particulate matter which cause deleterious effects to human health and the well-being of the environment [7]. Agro-wastes can also block drainage systems and canals ultimately leading to flood and erosion [6].

Compared to microbial fermentation, lactic acid synthesis by chemical means has several drawbacks due to its safety concern in the environment. In addition to being costly and dependence on other industries for raw materials, it also produces a racemic mixture of lactic acid when only one of the lactic acid isomers is required for a variety of uses [1,8]. The use of agricultural waste for lactic acid production can be viewed from both economic and environmental perspectives. From the economic perspective, the wastes may be used as inexpensive raw materials for the production of other improved compounds while lowering the cost of production, while from the environmental perspective, it abates the menace that may occur as a result of indiscriminate disposal [9].

Contrary to the chemical process, which results in compounds that are unsuitable for use in the production of food, beverages, and pharmaceuticals, microbial fermentation of LA yields highly optically pure enantiomers (e.g., over 98–99 % of L-LA) that are necessary for the creation of polylactic acid (PLA) [8]. In comparison to chemical synthesis, the fermentative approach has several additional benefits, including ease of use, the use of inexpensive renewable feedstocks, gentle pressure, and temperature conditions, decreased danger of contamination, low energy usage, and superior environmental performance [1,8]. The conversion of agro-wastes as a source of reducing sugar by indigenous isolates for lactic acid production will contribute towards the attainment of goal 7 (Agenda 2063) in the aspect of environmental sustainability and goals 6, 8, 9 and 12 (SDG 2030) in the areas of clean water and sanitation, industry, innovation, production and economic growth.

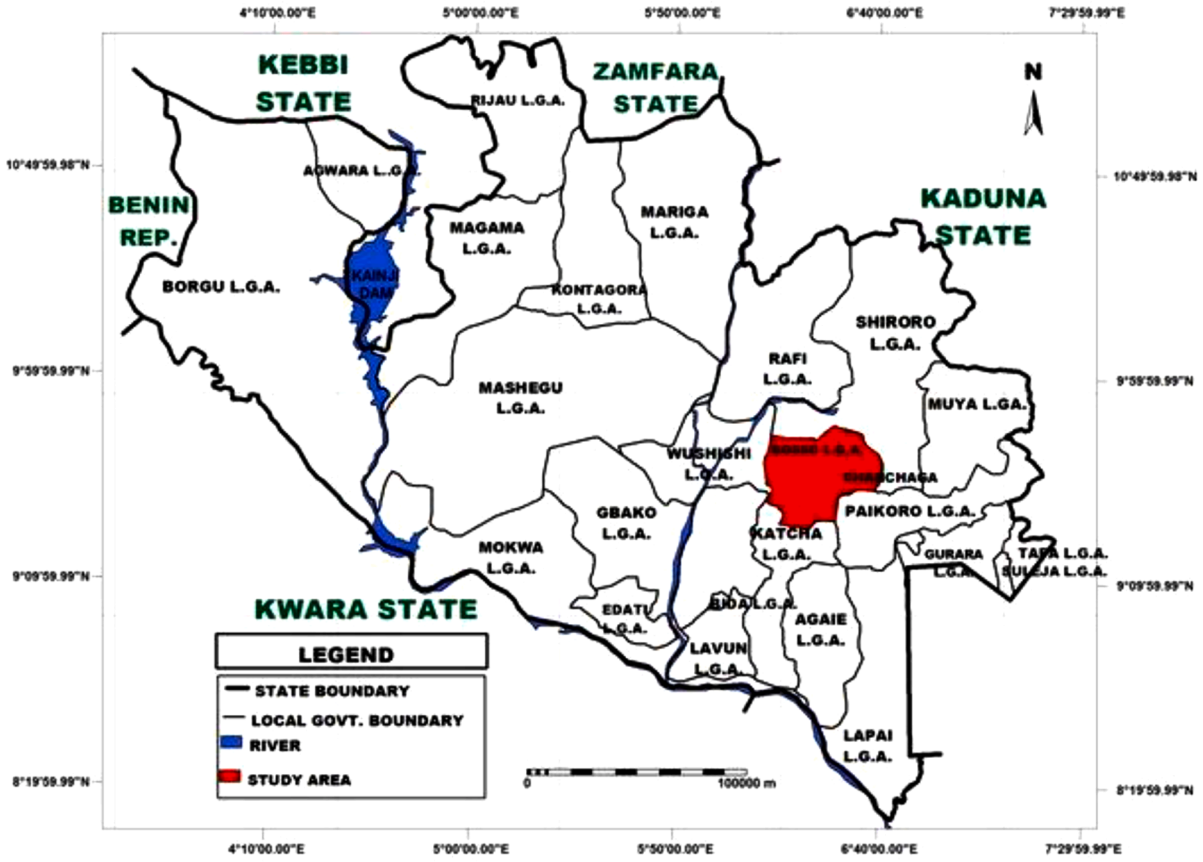

Fig. 1. Map of Niger State showing Bosso Local Government Area. Source: Department of Urban and Regional Planning, Federal University of Technology, Minna.

Therefore, the aim of this research was to produce lactic acid using reducing sugar from locally sourced lignocellulosic agro-wastes (corncob, sugarcane bagasse, plantain peduncle and groundnut shell).

Materials and method

Study area

This research was conducted in Bosso Local Government Area of Niger State, Nigeria (Fig. 1). The estimated population of Bosso was projected to be 208,212 in 2019 with a 2.5 % growth rate, and its land area is 1592 km^2. The geographical coordinates of Bosso Local Government are 9° 39′ 12" North and 6° 30′ 58" East.

Collection of samples

Soil samples were collected using a sterile spatula into sterile sample bottles from the same agricultural wastes (agro-wastes) dumpsite of local markets in Bosso Local Government Area, Minna Niger state. The samples were labelled according to each substrate, *viz*: plantain peduncles (PT), groundnut shell (GS), corncob (CC) and sugarcane bagasse (SB). Agro-wastes (plantain peduncle, groundnut shell, corncob and sugarcane bagasse) were collected aseptically from dumpsites of local markets in Bosso Local Government Area, Minna Nigeria and taken to the Microbiology laboratory of Federal University of Technology Minna, Nigeria for further analysis.

Media preparation

De Man Rogosa and Sharpe agar (MRS) was prepared by weighing 66.15 g of the agar, dissolved in a 2 L conical flask containing 1 L of distilled water, and heated until it completely dissolved. The medium was sterilized at 121 °C for 15 min and 15 psi pressure in an autoclave, set aside to cool to 40 °C, and then poured into sterile Petri dishes and allowed to solidify [10].

Isolation of lactic acid bacteria

Each soil sample labelled as PT, GS, CC, and SB obtained from the agro-wastes dumpsite were serially diluted up to a 10^{-7} dilution factor [11]. Inoculation was done by plating 0.5 mL aliquots from 10^{-2}, $10^{-4,}$ and 10^{-6} dilution factors of each sample using spread plate method with the aid of a spreader; at 37 °C the plates were incubated anaerobically for 48 h. The LAB characteristics colonies were purified by streaking on fresh nutrient agar (NA) plates and were also incubated anaerobically for 24 h. The purified isolates obtained from agro-wastes dumpsite were labelled accordingly. The isolates were stored on NA slants and kept in the refrigerator for further microbiological analysis [12].

Characterization and identification of lactic acid bacteria

The LAB was characterized *via* macroscopic observation, biochemical tests (i.e. catalase [13], oxidase [14], sugar fermentation test [15] and microscopy. Macroscopically, each unique isolate's colony elevation, colour, texture, form, and other distinctive and unique features were observed with unaided eyes as described by Getachew and Endale [16]. Gram's staining, however, was used for microscopic examination.

Pretreatment of agro-wastes substrates

The technique of Wischral et al. [17] was used to pretreat the agro-wastes (plantain peduncle, groundnut shell, corncob and sugarcane bagasse) and rinsed with clean running water, sun-dried for 5 days, and milled into powder. Twenty (20) g of sodium hydroxide (NaOH) was weighed and dispensed into 1 L of distilled water to obtain a NaOH solution. Ten grams (10 g) of the milled agro-wastes were weighed using a weighing balance and dispensed into different conical flasks, 100 mL of the NaOH solution was dispensed into the conical flasks containing 10 g of the substrates (this gave 10 % weight per volume) and were allowed to soak for 10 min before placing it inside a microwave (set at 40 % of 700, i.e. 280 w) to delignify the substrates for 5 min. Afterwards, the substrates were washed in a sieve several times to attain a neutral pH (7) before air drying.

Hydrolysis of agro-wastes substrates

The substrates were hydrolyzed using cellulase enzyme produced by Omonije et al. [18]. According to the methods described by Makut and Ekeleme [19], 20 g of each pretreated substrate was weighed and dispensed into 250 mL conical flasks, 100 mL of NaOH solution was added to each conical flask, and 20 mL of cellulase enzyme was also dispensed. The conical flasks were tightly corked and placed in a water bath for 48 h at 60 °C with frequent shaking to ensure uniform distribution of the enzyme. The hydrolysis was stopped after 48 h by increasing the water bath's temperature to 100 °C for 1 h to entirely suppress the enzyme's activity. The hydrolyzed substrates were sieved through a muslin cloth to obtain the hydrolysate, which was then filtered through a Whatman No. 1 filter.

Determination of reducing sugar concentration in the hydrolysate

The technique outlined by Khvedelidze et al. [20] was used to assess the concentration of reducing sugar in the hydrolysates. This was achieved by adding 1 mL of each hydrolysate to a test tube along with 1 mL of 3,5-Dinotrosalicyclic (DNS) indicator, making a blank as a reference, and heating the test tube at 100 °C for 5 min to detect colour change (from yellow to red). Thereafter, the samples were diluted with 9 mL of distilled water, and the samples' absorbance was calculated gravimetrically using a spectrophotometer set at 540 nm [18].

Submerged fermentation for production of lactic acid

Submerged fermentation was used to test the isolates for their ability to produce lactic acid in 50 mL conical flasks. The MRS medium was prepared by weighing the following components; Enzymatic digest of animal tissue (10 g), beef extract (10 g), yeast extract (5 g), sodium acetate (5 g), polysorbate 80 (1 g), dipotassium hydrogen phosphate (2 g), ammonium citrate (2 g), magnesium sulfate heptahydrate (0.05 g), and 20 g of agro-waste hydrolysates as a substitute for Dextrose (the fermentable carbohydrate), which is one of the components of MRS medium. A 20 mL of each fermentative media was dispensed into 50 mL conical flasks, tightly corked, and autoclaved for 30 min at 121 °C. After autoclaving, the media was allowed to cool, and 1 mL of inoculum was dispensed to their corresponding fermentative medium and then incubated for 24 h, an un-inoculated control without isolate was also incubated to serve as blank for spectrophotometric reading [20].

Determination of microbial growth on the hydrolysates

The growth of the bacteria was determined gravimetrically using a spectrophotometer (UV-Shamadzu UV spectrophotometer, Japan) after 24 h of incubation [21]. The isolate that produced the highest yield and fastest growth was subcultured on NA slant bottles and stored for further analysis.

Spectrophotometric determination of lactic acid produced

The quantity of lactic acid produced was measured according to the methods of Buruleanu et al. [21] with little modifications by dispensing 2 mL of culture containing lactic acid into clean test tubes and 2 mL of a 0.2 % solution of iron (III) chloride added and stirred. The concentrations were diluted 6-folds with deionized water and their absorbance was measured using a spectrophotometer (model UV-1800 Shamadzu UV spectrophotometer, Japan) at 390 nm. The absorbance (A) is proportional to the concentration of LA (c) in the range of 0.5 to 11 g/L. The equation of the calibration curve is given as $A = 0.1414c–0.0222$ [22]. Isolate with the most ability to produce LA was identified and characterized at molecular level and the gene responsible for LA production was determined.

Molecular identification of isolates

(i) **DNA extraction**

According to the methods of Tadesse et al. [23], the DNA was extracted. Briefly, cultures were grown on a shaker for 48 h at 28 °C using single colonies that had been established on medium and 1.5 mL of liquid media. Cultures were then centrifuged at 4600 g for 5 min. In 520 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), the pellets that were formed were re-dissolved. Then, 3 μL of Proteinase K (20 mg/mL) and 15 μL of 20 % SDS were added. A 10 % CTAB solution in 0.7 M NaCl and 100 μL of 5 M NaCl were added to the mixture and vortexed after it had been incubated at 37 °C for 1 h. The suspension was incubated for 10 min at 65 °C and then stored on ice for 15 min. After adding an equivalent volume of chloroform to isoamyl alcohol, the mixture was centrifuged at 7200 g for 20 min after 5 min of incubation on ice. The aqueous phase was then moved to a different tube, before isopropanol (1: 0.6) was added, and DNA precipitated at -20 °C for 16 h. Centrifuging DNA at 13,000 g for 10 min, washing it with 500 μL of 70 % ethanol, and letting it air dry for 3 h at room temperature (28 °C ± 2), before dissolving it in 50 μL of TE buffer to obtain the DNA.

(ii) **Polymerase chain reaction (PCR)**

According to the procedures outlined by Allaith et al. [24], the PCR was carried out. The PCR sequencing preparation cocktail comprised 10 μL of a 5x GoTaq colourless reaction, 3 μL of 25 mM $MgCl_2$, 1 μL of a 10 mM dNTPs mix, 1 μL of each 10 pmol 27F and 1525R primer, and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 μL with sterile distilled water 8 μL DNA template. A GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) was used to conduct the PCR. The PCR profile included an initial denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 50 °C for 60 s, and 72 °C for 1 min 30 sec; and a final termination at 72 °C for 10 min before chilling it at 4 °C.

(iii) **Integrity test**

The integrity of the amplified gene fragment was checked using the methods of Ghatak et al. [25] with little modifications on a 1.5 % agarose gel using the 1XTAE buffer. In a microwave, the suspension was heated to a boil for 5 min. After allowing the molten agarose to cool to 60 °C, it was dyed with 3 μL of 0.5 g/mL ethidium bromide, a substance that absorbs UV radiation but transfers the energy as

visible orange light. The casting tray's slots were fitted with combs, and molten agarose was then poured into the combs. For the wells, a 20 min period of gel solidification was required. The gel tank was filled with the 1XTAE buffer to just barely cover the gel. After loading the 100 bp DNA ladder into the first well, four microliters of each PCR product were added to two microliters (2 μL) of 10X blue gel loading dye, which provides the sample colour and density to make it simple to load into the wells and monitor the development of the gel. The gel was subjected to a 45 min, 120 V electrophoresis, which was then photographed and transilluminated in UV light. By evaluating the mobility of a 100 bp molecular weight ladder that was run in the gel alongside experimental samples, the sizes of the PCR products were calculated.

(iv) **Purification of amplified product**

After gel integrity, the amplified fragments were purified with the aid of ethanol in order to get rid of the PCR reagents [23]. Briefly, 7.6 μL of Na acetate (3 M) and 240 μL of 95 % ethanol were added to each Eppendorf tube containing 40 μL PCR amplified product, mixed thoroughly by vortexing and kept at 20 °C for 30 min. The mixture was then centrifuged for 10 min at 13,000 g and 4 °C followed by removal of supernatant (invert tube on trash once) after which the pellet was washed by adding 150 μL of 70 % ethanol and mixed before centrifuging for 15 min at 7500 g and chilled at 4 °C. Furthermore, all supernatant was eliminated by inverting the tubes on paper towels and allowing them to dry for 15 min in the fume hood at 20 °C. Prior to sequencing, the tubes were resuspended in 20 μL of sterile, distilled water and stored at 20 °C. The purified fragment was quantified using a nanodrop of model 2000 from thermos-scientific and tested on a 1.5 % Agarose gel run at 110 V for 1 h as before to confirm the existence of the purified product.

(v) **DNA sequencing analysis**

The DNA sequence was examined using the procedures described by Allaith et al. [24]. The BigDye terminator v3.1 cycle sequencing kit was used for sequencing the amplified fragments on an Applied Biosystems Genetic Analyzer 3130xl sequencer per the manufacturer's instructions. All genomic analyses were performed using MEGA 6 and Bio Edit software.

(vi) **Molecular identification of lactate dehydrogenase (D-Ldh) gene using genomic specific primers**

Molecular investigation of species-specific lactate dehydrogenase (d-ldh) gene genomic regions responsible for LA production in *Lactiplantibacillus plantarum* isolates was by simple PCR on the extracted DNA using genomic specific primers. Reaction cocktail used for all PCR per primer set included (Reagent volume μL) - 5X PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10 pM DNTP (0.25), 10 pM of each forward GGTGCCGATGTATACCAACA and backwards primer CATTGTCGCGGTTGAACAAG (0.25), 8000 U of Taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 μL template was added. PCR profile included an initial denaturing 5 min at 94°C, then 35 cycles of 94°C for 30 s, 48°C for 30 sec and 72°C for 20 s then terminate at 72°C for 10 min. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

Results

Cultural and morphological characteristics of the isolates

The macroscopic, cultural, and morphological properties of lactic acid bacteria isolated from the soils of different agro-wastes dumpsites were observed to be distinct. Several variations were observed in the colour, sizes, elevation and opacity of the colonies. All the isolates obtained were observed to exhibit three colors including creamy-shiny white (81.81 %), creamy-white (9.1 %), and off-white (9.1 %). The isolates exhibited two distinct cell sizes; 5 (45.45 %) isolates were small in size while 6 (54.54 %) of the isolates had large sizes. The colonies from corncob dumpsites appeared, creamy-shiny white and translucent with varying shapes and sizes. Colonies from sugarcane bagasse dumpsite, however, appeared creamy-white, dry, flat, and large. All isolates obtained from plantain peduncles and corncob appeared to be creamy-white with shiny surfaces.

Morphological, biochemical and sugar fermentation tests

The morphological characteristics of the isolates were ascertained based on microscopic examination following Gram's staining and observing the structural morphology under a light microscope. All the isolates observed were positive to Gram's reaction with a characteristic purple colouration. The isolates appeared to have varied structural arrangements; 5 (45.45 %) were rods, 2 (18.18 %) were cocci, the other 2 (18.18 %) were short rods and only one (GS_2) isolate appeared to be coccobacilli (Table S1).

Results from the biochemical tests are also presented in Table S1; all the isolates were negative to catalase and oxidase tests. For the sugar fermentation test however, only isolate PTO_3, ICC_2, and C_1 were positive to all the sugars (i.e. glucose, fructose, sucrose, lactose, mannose and rhamnose) tested with characteristics change in the colouration of the medium from red to yellow colour. While the remaining isolates showed varying abilities to utilize different sugar with varying characteristics in the production of acid and gas, isolate PTO_2 was observed not to produce gas in all the sugar it was cultivated in despite having the ability to utilize glucose and sucrose. Assessment of the various macroscopic cultural and morphological characteristics together with the microscopic morphology and sugar fermentation tests. The isolates obtained from the agro-wastes were all LAB (Table S1).

Molecular identity of the isolate with the best potential for lactic acid production

The agarose gel image of the isolate is shown in Fig. 2a and it shows that the size of the isolate is 1500 bp. The NCBI blast results showing sequence identity as compared to established sequences in the NCBI database along the 16s rRNA are presented in Table 1. The sequence of the isolate confirmed that it is 99 % similar to *Lactiplantibacillus plantarum*. The presence of 720 bp indicates a positive amplification in extracted DNA. The gel image of *Lactiplantibacillus plantarum* shows a positive amplification along the 16s rRNA to lactate dehydrogenase (d-ldh) gene, the gene that codes for lactic acid production (Fig. 2b, Table 1).

Reducing sugar concentration of the hydrolysates

The reducing sugar concentration of each hydrolysate was determined gravimetrically using spectrophotometry with 3,5-Dinotrosalicylic (DNS) as indicator. Upon measuring the reducing sugar concentration, groundnut shell was observed to have the highest value (23.04 mg/mL) followed by corncob (22.96 mg/mL), plantain peduncle (21.86 mg/mL), and sugarcane bagasse (21.45 mg/mL) respectively as presented in Fig. 3.

Lactic acid bacterial growths on the hydrolysate and lactic acid produced

The ability of different LAB to utilize different hydrolysates in this study was determined using the spectrophotometer at 540 nm. The highest absorbance reading was recorded by *Lactiplantibacillus plantarum* grown in fermentation broth containing plantain peduncles hydrolysate while the lowest absorbance value was recorded for *Lactobacillus mesenteroides* when grown in fermentation broth containing groundnut shell as carbon source (Table 2). The amount of lactic acid produced by the isolates with different substrates is summarized in (Table 2). The isolate *L. plantarum* grown in plantain peduncle hydrolysate was observed to give the highest lactic acid yield of 6.21 g/L while the lowest yield of lactic acid (4.32 g/L) was produced by *L. casei* that utilized groundnut shell as carbon source. A positive correlation ($p < 0.05$) was observed between the microbial growth (absorbance) and the amount of lactic acid produced.

Discussion

Lactic acid may be produced by a wide range of microorganisms known as lactic acid bacteria from a number of sources. The eleven (11) LAB isolates were macroscopically examined on MRS agar, and it was discovered that the colonies of these bacteria had a creamy-white appearance, while some were shiny and others dried; some of the isolates were raised and others flat; some appeared to be opaque and others translucent with varying cell sizes and forms. Similarly, Bakar et al. [26] isolated 20 strains of bacteria from 8 soil samples, among which 9 grew on MRS media whose colonies were white or cream, convex, with smooth elevation and entire margins with varying cell sizes.

The microscopic analysis of the isolates shows that LAB could be cocci, rod, or coccobacilli (short rods) in shape, and are mostly Gram-positive bacteria. Two families make up the shape of LAB cells, these are; (a) Lactobacillaceae, which are rod-shaped and include the genera *Lactobacillus* and *Bifidobacterium*, and (b) Streptococcaceae, which are round and include the genera *Streptococcus, Leuconostoc*, and *Pediococcus*. The oxidase test yielded negative results for all of the isolates, characterized by the absence of dark purple discoloration on the filter paper after the addition of drops of 1 % tetramethyl p-phenylene diamine dihydrochloride. This could be because the isolates had no cytochrome C, which clearly shows that they are anaerobic organisms and cannot use free oxygen in

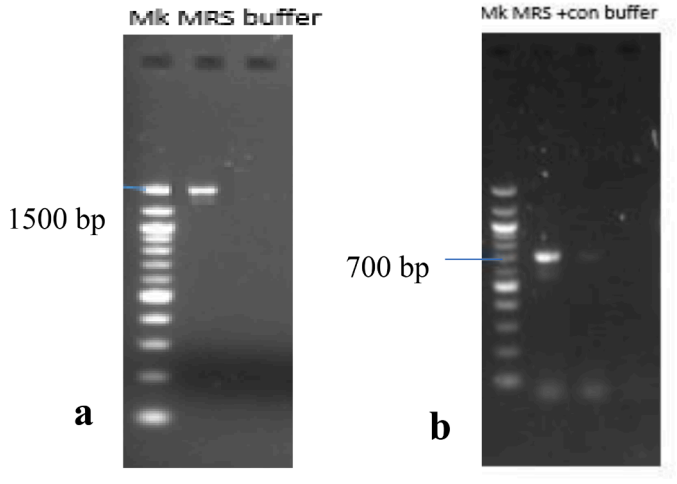

Fig. 2. (a) Gel image of isolate (b) Agarose gel showing the positive amplification of lactate dehydrogenase (d-ldh) *Gene* amplified from DNA isolated from cultured bacteria.

Table 1
NCBI blast results showing sequence identity along the 16s rRNA.

Sample ID	Scientific name	Max score	Total score	Query cover	E value	% Identity	Accession number
MRS	*Lactiplantibacillus plantarum*	2569	2569	99 %	0	99.37 %	OM510300
MRS	*Lactiplantibacillus plantarum* lactate dehydrogenase (d-ldh) gene	1315	1315	99 %	0	99.86 %	CP053912.1

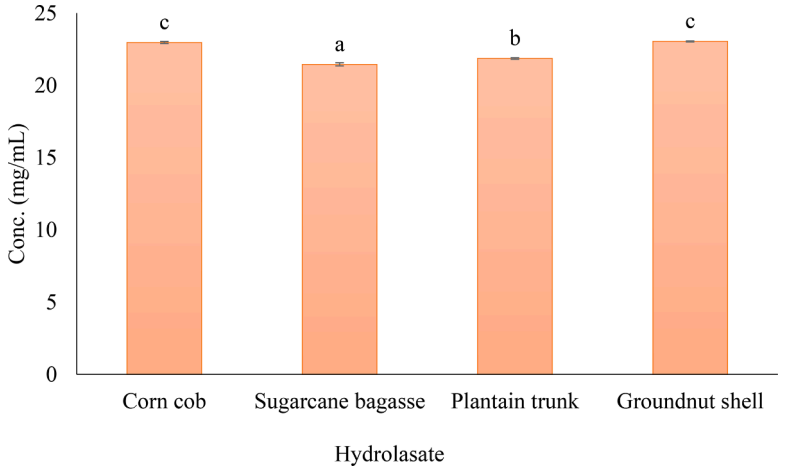

Fig. 3. The reducing sugar concentration of the hydrolysates.

Table 2
Lactic acid bacterial growth and yield of lactic acid.

Isolates	Microbial growth (absorbance)	Lactic acid (g/L)	Correlation (r)
Lactobacillus brevis	0.90 ± 0.01^{e}	5.08 ± 0.12^{b}	
Streptococcus uberis	0.89 ± 0.03^{de}	4.76 ± 0.06^{b}	
Leuconostoc citrovorum	0.63 ± 0.01^{c}	4.54 ± 0.03^{a}	
Lactobacillus casei	0.64 ± 0.01^{c}	4.86 ± 0.12^{ab}	
Lactobacillus plantarum	0.63 ± 0.02^{c}	4.61 ± 0.05^{ab}	
Lactobacillus plantarum	1.96 ± 0.02^{g}	6.21 ± 0.02^{c}	+ 0.888
Leuconostoc citrovorum	0.90 ± 0.01^{e}	5.04 ± 0.09^{b}	
Lactobacillus acidophilus	1.03 ± 0.03^{f}	5.26 ± 0.11^{b}	
Lactobacillus brevis	0.81 ± 0.02^{d}	5.41 ± 0.10^{b}	
Leuconostoc mesenteroides	0.47 ± 0.00^{a}	4.84 ± 0.03^{ab}	
Lactobacillus casei	0.55 ± 0.01^{b}	4.32 ± 0.05^{a}	

NB: Alphabets (in superscripts) with same letter are not significant ($p > 0.05$) while different letters are significant ($p < 0.05$).

their energy metabolism [14].

In this study, all the isolates lack the ability to mediate the decomposition of H_2O_2 to produce oxygen, as no bubbles were seen, indicating that they are catalase-negative. This observation is consistent with the findings of Nguyen et al. [27], where all the LAB isolated from the soil were catalase-negative; thus, it has been established that LAB do not produce catalase. The ability of the isolates to generate gas was examined in order to establish the kind of fermentation that the LAB isolate may undertake. Their ability to use carbohydrates and create lactic acid was established in the sugar fermentation test. *Streptococcus, Pediococcus*, and *Lactobacillus* are examples of LAB that undergo homo-fermentation, while *Leuconostoc* and several species of *Lactobacillus* are examples of LAB that undergo hetero-fermentation [28].

The agro-wastes (plantain peduncle, groundnut shell, corncob and sugarcane bagasse) used as substrates for lactic acid fermentation showed varying concentrations of reducing sugars with groundnut shell having a higher reducing sugar concentration of 23.04 mg/mL while corncob, plantain peduncle and sugarcane bagasse had reducing sugar concentrations of 22.96 mg/mL, 21.86 mg/mL, and 21.45 mg/mL respectively. This could be due to the difference in their chemical composition as stated by Screenath et al. (2014).

The microbial growth on the hydrolysates were in varying degrees. One of the isolates from plantain peduncle dumpsite identified as *Lactiplantibacillus plantarum*, had a higher absorbance value (i.e. it grew more rapidly) when grown on fermentation medium containing plantain peduncle hydrolysates while the other isolates grew moderately and had different absorbance values on other agro-wastes hydrolysates. Given the fastidious nature of the isolate, it is possible that the hydrolysate contained all the nutrients the

isolate required to grow quickly. This finding agrees with that of Screenath et al. (2014). The isolates produced varying amounts of lactic acid, with *L. plantarum* yielding the most, with an absorbance value of 0.9006 (6.21 g/L of lactic acid) after 48 h. Thus, the isolate from plantain peduncle dumpsite identified as *Lactiplantibacillus plantarum,* which had the best ability to produce lactic acid, was selected.

Conclusion

The results of this study demonstrated that plantain peduncle may be a cheaper source of reducing sugar for lactic acid production and also act as a means of preventing environmental pollution since it prevents the agro-waste from being discarded indiscriminately into the environment. This plantain peduncle is in abundance in Nigeria and has been left untapped, constituting nuisance to the environment. The results of this study also established that *Lactiplantibacillus plantarum* from plantain peduncle dumpsite was the most effective isolate for producing lactic acid because of its rapid development in the fermentation broth. The findings of this research can be useful to African leaders in making informed decisions and policies to tap the unearthed enormous agro-wastes potential of waste to wealth. This will enhance economic development, industrialization, reduction in hunger and poverty, as well as result in cleaner environment.

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CRediT authorship contribution statement

Oluwafemi Adebayo Oyewole: Conceptualization, Supervision, Writing – review & editing. **Japhet Gaius Yakubu:** Investigation, Writing – review & editing. **Justina Kalu:** Investigation, Writing – review & editing. **Maryam Temitola Abdulfatah:** Investigation. **Olabisi Peter Abioye:** Writing – review & editing. **Olalekan David Adeniyi:** Methodology. **Evans Chidi Egwim:** Methodology, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2023.e01915.

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