Original Research Article

PHYLOGENETIC IDENTIFICATION AND SOME PHYSIOLOGICAL PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM THE ORAL CAVITIES OF SOME SELECTED STUDENTS FROM UNIVERSITY OF IBADAN, NIGERIA.

ABSTRACT.

Lactic Acid Bacteria (LAB) have been reported to be a component of the human and animal microbiome, functioning as probiotics. Their capacity to stick to the epithelial or mucosal surface is mostly dependent on receptor specific binding, charge, and hydrophobicity interaction. Much work have been done on the hydrophobicity and some physiological properties of LAB from different sources but there is a dearth of knowledge on those of the oral cavity, hence this study.

Oral samples were collected from respondents from different areas of the University Of Ibadan with sterile swab stick and were brought to the microbiology laboratory of the University of Ibadan for analysis. The isolates were subjected to morphological, biochemical, molecular and physiological tests using standard procedures.

In this study, nine colonies of LAB were isolated and characterized from oral samples of respondents from the University of Ibadan. They were identified as, *Pedicoccus acidilactici, Leuconostoc lactis, Leuconostoc mesenteroides, Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarium,* and *Lactobacillus casei*. All the isolates inhibited the growth of the four pathogens used, survived at 4% concentration of Nacl, $MgSO_4$, KNO_3, $NaNO_3$ and $MNSO_4$ and 3% bile salts used. They exhibited low surface hydrophobicity, survived in the acidic and temperatures of the media used.

Keywords: Hydrophobicity, Lactic Acid Bacteria, Physiological properties and Probiotics

1.0 INTRODUCTION

Lactic Acid Bacteria (LAB) became more well-known in the early twentieth century (Liu *et al.,* 2014). Lactic acid bacteria are phylogenetically related bacteria that have similar morphological, metabolic, and physiological characteristics. This group includes Gram-positive, non-sporulating, non-respiring cocci or rods that release lactic acid as a significant end product of carbohydrate fermentation. Lactobacillus, Leuconostoc, Pediococcus, and Streptococcus are commonly recognized as a core group of four genera. (Sun *et al.,* 2014).

Phenotypic approaches have been most often utilized in the identification of LAB (Sandberg *et al.,* 2019). The identification of short 16S rDNA sequences is now used as a simple method for detecting the species of lactic acid bacteria isolates (Abbas Ahmadi *et al.,* 2014). Lactic acid bacteria are classified into several genera based on their capacity to grow in high salt concentrations, acid or alkaline tolerance, morphology, glucose fermentation method, growth at different temperatures, and the configuration of the lactic acid produced. (König & Fröhlich, 2017).

Lactic Acid Bacteria (LAB) are gram-positive rods and cocci that do not generate spores and are non-motile. Only fermentation, generally of sugars, may provide ATP to the LAB. Lactic acid bacteria thrive in anaerobic environments because they do not require oxygen for energy synthesis, but they can also thrive in the presence of oxygen. These organisms are anaerobes that can survive in the presence of oxygen (König & Fröhlich, 2017). Arena *et al.* (2017) reported that Lactic acid bacteria can be found in the intestines and the mouth of both humans and animals.

Enzymes, vitamins, antioxidants, and bacteriocins are all produced by LAB and are crucial processes for the metabolism and detoxification of exogenous chemicals that enter the body. (Li *et al.*, 2018).The serum antibodies IgG and secretory IgA and TgM have been discovered to control intestinal disorders, and boosting of immune response (Rezende *et al.,* 2018).

The mouth microbiota coexists with the host in healthy persons who follow correct nutritional and dental hygiene practices, preventing pathogen colonization and contributing to host physiology (Hezel, 2015). The most prevalent occupants of the oral cavity are bacteria. The majority of organisms in healthy persons belong to the bacterial phylum. The different types of bacteria are Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Fusobacteria (Verma *et al.,* 2018).

Natural microflora exists in the oral cavity, just as it does in other areas of the gastrointestinal tract. It possesses a variety of characteristics that distinguish it as a distinct microbial species. Teeth have hard, non-shedding surfaces that allow enormous numbers of germs to accumulate (dental plague), especially in stagnant places (Marsh, *et al.,* 2016). Another notable aspect is that the oral cavity is constantly soaked in saliva, which has a significant impact on the mouth's ecology. Many bacteria thrive in saliva, which has a pH range of 6.75-7.25. Salivary components affect oral microbes through one of four mechanisms: aggregating microbes to aid their removal from the mouth, adsorbing to tooth surfaces to form an

acquired pellide to which microorganisms can attach, serving as a primary source of nutrients, and medicating microbial inhibition or killing (Gevers *et al.,* 2012). The gingival crevicular fluid (GCF), which runs through the juctional epithelium and gives nutrients to bacteria in the gingival fissure as well as carrying host immunological components that play a key role in maintaining the microbiota there, is similar to saliva (Benítez-Páez, 2014)

Both receptor-specific binding and hydrophobic interaction are involved in adherence to an epithelial surface. Cell surface hydrophobicity (CSH), as determined by the Salt Aggregation Test (SAT), contact angle, and adherence to xylene are all prevalent in LAB (Cao, 2017). Due to the hydrophilic moieties that surround microbial surfaces, most organisms come in touch with water and are always in the aqueous phase (Nayarisseri *et al.,* 2019). Charged groups such as carboxyl, phosphate, amino, and guanidyl groups, as well as the non-charged hydroxyl group, make up the hydrophilic sites of bacterium cell walls, while lipids and lipopolysaccharides make up the hydrophobic sites (Kim *et al.*, 2021). Hydrophobic responses are known to have a role in microbe adhesion to a variety of surfaces in the environment. The hydrophobic characteristic of microbes is linked to their partitioning activities at interfaces, which explains their adherence to surfaces, marine sediment, and one another, as well as their growth on hydrophobic compounds (Marshall, 2013). Microbial cell surface hydrophobicity varies from organism to organism and strain to strain, according to (Choi *et al.,* 2015) and is regulated by the growth medium, bacterial age, growth temperature, pH, ionic strength, and cell densities.

The purpose of this research is to identify and analyze for some physiological properties of Lactic acid bacteria isolated from the oral cavities of selected university of Ibadan students in southwestern Nigeria.

2. MATERIALS AND METHODS.

2.1 Collection of Oral Samples

Oral samples were collected from some randomly selected male and female students of the University of Ibadan. The samples were obtained using sterile swab sticks and brought to the University of Ibadan's Department of Microbiology laboratory for prompt examination.

2.2 Indicator Organisms

Indicator organisms were collected from the collection Desks, Medical Microbiology Laboratory, University College Hospital (UCH), University of Ibadan.

2.3 Culture Medium

Lactic acid bacteria species were isolated using De Man Rogosa and Sharpe (MRS) medium. (DE Man Rogosa *et al.*, 1960). The weighing balance (Microwa, Swiss Electric Balance) was used to weigh the components of the isolation medium, 500ml distilled water was added to the MRS agar ingredient in 10 litre Erlenmeyer flask. To completely dissolve the component, the solution in the flask was homogenized on an electric hot plate for 10 minutes. For the purpose of this investigation, the dissolved medium was sterilized in an autoclave at 1 atm for 15 minutes and allowed to cool before use, and the pH was adjusted to 5.5 with dilute HCl using an electric pH meter (Christian, 1970).

2.4 Isolation Procedure

For Lactic Acid Bacteria isolation, approximately 10ml of sterilized MRS agar medium was aseptically dispensed into sterile petri dishes. After allowing the MRS agar to solidify in the sterile petri dishes, the samples collected on the sterile swab sticks were streaked over the hardened MRS agar surfaces in the various sterile Petri dishes. The petri dishes were anaerobically incubated for 48 hours at 30°C. The plates were examined for bacterial growth after the incubation period, and representative colonies were chosen at random. To obtain pure cultures, isolates were sub-cultured and streaked repeatedly. The colonial shape and cellular properties of the different colonies were examined.

2.5 Culture Preservation

Pure LAB isolate cultures were sub-cultured into MRS broth containing 12 %v/v glycerol and incubated at 30°C until visible growth emerged. To prevent further development, the culture was held at 4 ^{o} Celsius. The isolates were placed into fresh maintenance medium with the same composition at 4week intervals. Isolates of indicator organisms were kept in the same way, on nutrient broth, and incubated at 35^{o}C until growth was observable. For later use, the stock cultures were kept at -4^{o}C.

2.6 Lactic Acid Bacteria isolation and identification

Isolates were characterized using microscopic, macroscopic, physiological, biochemical, and molecular tests. Unless otherwise noted, all assays employed one milliliter of broth culture of each isolate.

2.7 Morphological Characterization

The type of growth, form, elevation, size, propagation, and consistency of the bacteria were all evaluated.

2.8 Biochemical Characterization

2.8.1 Gram's Technique for Staining

The pure isolates were dyed according to Norris and Ribbond's procedures (1971). A tiny smear of the isolates was made on clean glass slides and heat-fixed by flame. Two drops of 5% Crystal violet are applied to the smear for one minute. The crystal violet drops were washed in water before being stained with Gram's Iodine solution for one minute. By soaking the slide in alcohol until no more violet colouring was apparent, the stain was removed. Two drops of 2% Safranin were applied for 10 seconds before being rinsed and wiped dry with filter paper. Oil immersion objective was used for observation. Gram-positive bacteria had a purple coloration, whereas Gram-negative bacteria had a pinkish coloration. The varied shapes and arrangements of bacterium cells are also visible with this staining approach.

2.8.2 Catalase Test

The streaked bacterial isolates were cultured for 18 hours on MRS agar plates. Each plate received a drop of freshly made 3% hydrogen peroxide solution (Seeley and Van Demark, 1972). A catalase positive reaction is shown by the evolution of gas white foam. An unfavorable reaction is indicated by the lack of foam.

2.8.3 Motility

The organisms were cultivated for 18 hours at 30°C in MRS broth. Following the incubation period, very few drops of broth were poured on a clean glass and examined using the oil immersion lens on the microscope. (Seeley and Van Demark, 1972).

2.8.4 Oxidase Test

For this test, Whatman (number 1) filter paper was used. A few drops of oxidase reagents (1% aqueous tetramethyl-p-phenylenediamine dihydrocloride) were applied to the whatman paper using a sterilized wire loop. The wire loop was sterilized once more and used to touch the test isolate colony before being transferred to the reagent location on the Whatman paper. A good reaction was indicated by the formation of a very deep purple colour within 10 seconds, whereas a negative reaction was shown by the absence of a deep coloration (Seeley and Van Demark, 1972).

2.8.5 Starch Hydrolysis

An equimolar amount of soluble starch was prepared and added to MRS and Nutrient agar without glucose or meat extract before being poured into sterile plates to make a 1% soluble starch. On a dry plate, single streaks of cultures were produced and cultivated for 48 hours at 30°C. The plates were flooded with Gram's iodine after incubation. When iodine was added to un-hydrolyzed starch, it turned blue. Starch hydrolysis by the culture was shown by clear zones around the growing region (Seeley and Van Demark, 1972).

2.8.6 Hydrogen Sulfide Test

The ability to create hydrogen sulfide from organic molecules is determined by this. The isolates were placed in sterile MRS and Nutrent broth tubes (no sulphur compound was introduced). The inoculation media was then incubated at 35-37^{o}C for 5 days with a lead acetate paper strip inserted in the tube but above the medium. The black colour of the filter strips indicated the presence of H_2S.

2.8.7 Nitrate Reduction Test

Nitrate peptone water was used, which was made up of peptone water and 1% potassium nitrate. Each of the 27 screw-capped test tubes contained a five milliliter amount of the medium. Before inoculating with the isolates, each tube's contents were sterilized (121^{o}C for 15 minutes) and allowed to cool. Uninoculated tubes served as controls. For four days, the tubes were incubated at 30°C. By adding 0.5ml of 0.6 % dimethylnaphthylamine in 5.0ml acetic acid to each tube, the ability of the isolates to convert nitrate to nitrite, ammonia, or free nitrogen was measured. A positive result and the production of nitrogen gas was indicated by the development of a red coloration (Payne, 1973).

2.8.8 Voges- Proskauer

After two days of incubation at 30^{o}C, the isolates were grown in methyl red broth with 1ml of 6-alpha-napthol solution and 1ml of 10% NaOH (Sodium hydroxides). The purpose of the test is to see if the organisms can produce acetyl methyl carbinol from acid after producing acid from glucose. If the result is negative after 5 minutes, a pale pink coloration may appear for up to 1 hour to check for a slow reaction.

2.8.9 Spore Staining Technique

The technique of Schaeffer - Futton staining was used. On clean glass slides, smears of pure cultures were created. Malachite green reagent was pumped into the smears. The slides were heated in boiling water and then allowed to cool for 5 minutes. The slides were carefully rinsed under slow-running tap water, then counter-stained for 1 minute with Safranin, rinsed thoroughly, and examined under the microscope. Spores retain the initial stain's green color and remain green, whereas vegetative cells stain pink.

2.8.10 Production of Ammonium from Arginine

Instead of ammonium citrate, ammonia was produced from arginine broth without glucose and meat extract, but with 0.3 % arginine and 0.2 % sodium citrate. As a control, 18-24 hours of MRS broth without arginine were used. Lactic acid bacteria cultures were inoculated into 10ml of each broth in a test tube and incubated for four days at 37 ° C. A drop of Nessler's reagent was added to the culture on a plate after it had been incubated. Cultures that produced a yellow or orange colour as compared to cultures inoculated with the same amount of arginine produced ammonia (Doring, 1988).

2.8.11 Homofermentative and Heterofermentative Test

20 ml of MRS broth were poured into McCartney bottles, sterilized, and inoculated twice. Each bottle's surface was sealed with sterile agar seal. The presence of gas was detected by pressing agar seals into the tubes. Bottles that had not been inoculated served as a control (Gibson and Abdel- Malek, 1945).

2.8.12 Methyl Red Test

How to prepare glucose phosphate peptone broth was reported by Harringan and MacCane (1996). In sterilized screw cap tubes, 10 mL of broth were dispensed. The test organisms were then injected and incubated for 2–5 days at 30°C. After incubation, a few drops of methyl red indicator were applied to the culture, and the subsequent definite red colour was considered positive.

2.9 Molecular characterization of the isolated microorganisms

Bacterial isolates were subjected to polymerase chain reaction (PCR) analysis for identification. Bacterial DNA was extracted using the cTAB technique, then processed with proteinase K and RNAase (20 mg/ml) before being PCR-amplified using a universal primer set flanking the 16S ribosomal rRNA on an Applied Biosystems Thermalcycler (a

GeneAmp 9700). To evaluate the purity and purification of the amplified fragments, they were put into a 1.5 % gel. The amplified fragments were sequenced using the Big Dye terminator v3.1 cycle sequencing kit and the Genetic analyzer model 3130xl sequencer according to the manufacturer's instructions. For identification, the sequences were run through the BLAST search software on the NCBI website.

2.9.1 Phylogenetic analysis

The isolated microorganisms' sequences were evaluated and compared to other similar sequences previously submitted to the NCBI, and they were aligned using Cluster X 2.1. (Thomson *et al.,* 1997). Molecular Evolutionary Genetics Analysis (MEGA 7.0) software was used to assess the phylogenetic relationship between the microorganisms (Tamura *et al.,* 2013; Kumar *et al.,* 2016). The neighbor-joining tree was constructed using the Kimura 2-parameter distances and 1000 replicates of bootstrapping.

2.10 PHYSIOLOGICAL PROPERTIES

2.10.1 Growth at different temperature

Tubes containing 10ml of MRS and Nutrent broth were inoculated with LAB and other bacteria isolates respectively and incubated at 15^{o}C, 30^{o}C, 37^{o}C, 40^{o}C, and 45^{o}C for 4 days (Gibson and Abdel- Malek, 1945).

2.10.2 Growth in the presence of 4% sodium chloride

20ml of MRS and Nutrient broth containing 4% NaCl, were dispensed into screw capped tubes, sterilized and inoculated with LAB and other bacteria isolates respectively. The tubes were incubated at 30^{o}C for 4 days. Increased turbidity of media was recorded as positive for growth. Un-inoculated tubes served as control (Rogosa and Sharpe, 1959).

2.10.3 Acid resistance test

The pH values of the different MRS broth media were adjusted to pH 2.0, 3.0, 4.0 and 5.0 respectively using 0.1M HCl solution. 10mls of the media were distributed into tubes before autoclaving. After autoclaving the pH value of the medium were rechecked and then the media were inoculated with the test cultures before incubation at 30^{o}C for 48 – 120 hours. Turbidity of the broth compared with the un-inoculated controls were used as indicator of growth of the cultures (Feltham *et al.,* 1978).

2.10.4 Screening for the Antimicrobial activity of the Test Isolates

The agar well diffusion method was used (Schillinger and Lucke, 1989). The indicator bacteria used were the twelve isolates and four enteric bacteria. Serial dilution of each entero-pathogen was carried out. 1ml of each was taken from the 10^{-6} dilution. Using pour plate method, the samples were dispensed into the sterile plates. The nutrient agar was sterilized at 121^{o}C for 15minutes using an autoclave. It was then allow to cool before pouring into the plates aseptically, for even distribution, the plate was swirled and allowed to gel. Wells, with a diameter of 5mm were then cut in the agar using a sterile cork borer. With a sterile pipette, 3 drops of the culture supernatants were introduced into the wells. The inoculated dished were then incubated for possible zone of inhibition.

2.10.5 Bile Tolerance Test

The procedure for determining bile tolerance was identical to that described by Gilliland *et al* (1984). Lactic acid bacteria strains were cultured overnight in MRS broth, then 0.1ml of the culture suspension was injected into tubes containing 10ml MRS broth with or without 0.3 percent bile salt (which acted as controls).The inoculated tubes were incubated at 37^{o}C. Each LAB strain in each treatment received three tests, each with a duplicate. The absorbance at 540nm was measured using a spectrophotometer (DV-65, Bechman, Fullerton, USA) for 6 hours in both the bile salt and control solutions to monitor growth .The difference in hours between the control and bile culture was interpreted as a delay in growth (bile tolerance) caused by bile inhibition (Chateau *et al.,* 1994).

2.10.6 Hydrophobicity Assays

MRS broth was used to culture the bacteria. Under anaerobic circumstances, the bacteria were cultured for 24 hours at 37 degrees Celsius. The strains were harvested and centrifuged at 4000r/min for 5 minutes at 5^{o}C with a centrifuge model 0406-2, washed twice with 50mm K_2HPO_4 (pH 6.5) buffer, and lastly re-suspended in the same buffer with the value set to 1.0 using the buffer's control. 3ml bacteria suspensions were vortexed for 120 seconds after 0.6ml xylene was introduced. The supernatant was removed and the residue was measured at 540nm. The decrease in aqueous phase absorbance was used to compute the cell surface hydrophobicity (H%), which was estimated using the formula below.

$$H\% = [(A0 – A) / A0] \times 100$$

Where A0 and A were absorbance value before and after extraction with xylene, respectively. (Anaerobic, 2006. 03.001) Elsevier Ltd (Whitely *et al.*, 2015).

2.11 **Statistical analysis**

Results were presented as Mean ± SEM.

3. RESULTS AND DISCUSSION

A total of nine isolates of Lactic Acid Bacteria (LAB) were randomly isolated from the 'Oral' samples collected from different respondents from the University of Ibadan. The isolates were subjected to Morphological, Biochemical, Molecular and Physiological Tests. Detailed results of the morphological and biochemical characteristics of the isolates are shown in (Tables 1a, 1b). Microscopic examination showed that some of the isolates have small colonies in which the length of the rod varied between medium short rods to relatively long rods while other may be spherical or cocci. The Grams reaction test showed that all the isolates were gram positive and did not produce endospore. They gave negative result for Catalase, Indole, Hydrogen sulfide production, oxidase, Motility, Starch hydrolysis and Voges Proskauer test. They are all methyl red positive. All the organisms could not produce ammonia from arginine with the exception of *Lactobacillus plantarium.* Phylogenetic analysis revealed the identification of the sequenced species with a confidence level of at least 99.25 % revealing that they clustered with the most closely related reference sequence retrieved from NCBI data, as shown in Figure 1 and table 2. The tree split into two big clades, the larger of which had many branches which included the, A2- *Pediococcus acidilactici* with accession number OM763689, A3- *Leuconostoc lactis* with accession number OM763690, A4- *Leuconostoc mesenteroides* with accession number OM763691, A6- *Lactobacillus acidophilus* with accession number OM763692, A7- *Lactobacillus reuteri* with accession number OM763693, A8 - *Lactobacillus brevis* with accession number OM763694, A9- *Lactobacillus fermentum* with accession number OM763695, A10- *Lactobacillus plantarum* with accession number OM763696 and A11- *Lactobacillus casei* with accession number OM763697 reference sequences. The second claude, on the other hand, is made up of a BS41 srain branch that formed a close cluster with the *Pseudomonas aeruginosa* reference sequence.

In this study, nine colonies of Lactic Acid Bacteria (LAB) were isolated and characterized from oral samples of respondents from the University of Ibadan. These Lactic Acid Bacteria include, *Pedicoccus acidilactici, Leuconostoc lactis, Leuconostoc mesenteroides,*

Lactobacillus acidophilus, Lactobacillus reuteri, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarium, and *Lactobacillus casei*. This result is in support of the findings of Arena *et al.* (2017), that LAB are parts of oral flora.

Table 1a.

Table 2.

Figure 1.

All the Lactic Acid Bacteria grew at 15^{o}C except *Pediococcus acidilactici, Leuconostoc lactis* and *Lactobacillus acidophilus* while all the isolates grew at 30 ^{o}C, 37 ^{o}C, and 41 ^{o}C as shown in Table 1b. This is in support of the findings in Prescott *et al.* (2020) that LAB are mesophilic organisms which grow well at temperature range of 20^{o}C to 40^{o}C. The more active bacterial enzymes are, the higher the rate of metabolic activities and this could have been responsible for the growths at these temperatures.

Table 1b.

Table 3, shows the growth of the various isolates in 4% Sodium chloride, Magnesium sulphate, Potassium nitrate, Sodium nitrate and Manganese sulphate. For the growth of the isolates in NaCl, *Lactobacillus casei* had the highest growth with mean growth of 1.201±0.01 while *Pediococcus acidilactici,* had the lowest mean growth value of 0.687±0.01. For the growth of isolates in MgSO4, *Lactobacillus casei* had the highest mean growth of 1.196±0.03 while *Pediococcus acidilactici,* had the lowest mean growth value of 0.750±0.01. For the growth of isolates in KNO_3, *Lactobacillus casei* had the highest growth with mean growth of 1.206±0.01 while *Pediococcus acidilactici,* had the lowest mean growth value of 0.866±0.02. For the growth of isolates in $NaNO_3$, *Lactobacillus casei* had the highest growth with mean growth of 1.204±0.03 while isolate *Lactobacillus reuteri* had the lowest mean growth value of 0.324±0.02. For the growth of isolates in $MnSO_4$, *Lactobacillus fermentum* had the highest growth with mean growth of 0.814±0.02 while *Pediococcus acidilactici,* had the lowest mean growth value of 0.345±0.01.The mean growth value of the control is 0.152±0.01.

All the isolates were able to grow well at 4% concentration of $MgS0_4$, $NaNO_3$, NaCl and $MnSO_4$ because magnesium, sodium and potassium are components of many chemically defined media for bacteria growth while Manganese is a component of micronutrients as reported by Prescott *et al.,* (2020).

Table 3

The growth of the Lactic Acid Bacteria (LAB) isolate at different acidic pH concentration is shown in Table 4. All the LAB grew at pH (2.0, 3.0, 4.0, 5.0). In the overall, the highest growth was observed at pH 2.0 with *Lactobacillus casei* at mean growth of 0.913±0.04 while the lowest value of growth was observed with *Lactobacillus plantarium* at pH 5.0 with mean growth of 0.503±0.01. It was generally observed that all the isolates were able to survive and grow well in different media at different pH of which the highest growth was observed between pH 2 and 3 which is in line with the findings of Krieger-Weber *et al., (*2020) who reported that generally at pH below 3.5, species of *Pediococcus* and *Lactobacillus* grow well.

Table 4.

The antagonistic activities of Lactic Acid bacteria isolates against certain indicator organisms were examined using the agar diffusion method, as shown in Table 5. All the LAB isolates produced compounds with antagonistic activity toward all the indicator organisms used in this study. *Lactobacillus reuteri,* with a mean value of 16.83±1.424, had the highest zone of inhibition against *Staphilococcus aureus,* whereas *Leuconostoc mesenteroides,* with a mean value of 9.20±0.611, had the lowest zone of inhibition against *Escherichia coli.* This is in conformity with the findings of Garcia-Gutierrez *et al.* (2019) that Lactic Acid Bacteria help to suppress undesirable bacteria by helping to maintain an antibacterial and antifungal environment. These LAB achieves this because they produce large quantities of acetic acid, hydrogen peroxide, diacetyl, and bacteriocin, which inhibits growth of pathogenic bacteria even when prolonged antibiotics therapy has failed (Admassie, 2018).

Table 5.

The bile tolerance values of Lactic Acid Bacteria isolates from oral samples of respondent from different sections of the University of Ibadan is shown in Table 6. *Leuconostoc lactis,* had the highest bile tolerance with the value of - 0.047 while *Pedicoccus acidilactici*, had the lowest bile tolerance value 0.16. In the overall, all the LAB isolates exhibited bile tolerance in the test. The result of the comparison of the growth rates of Lactic Acid Bacteria in the control and 3% bile salt for six hours revealed that Bile salt had some initial inhibitory effect on the growth of LAB isolates after which, LAB was able to grow. This explains the reason why LAB forms part of the natural flora of the intestine.

Table 6.

For the hydrophobicity test, the results showed that *Lactobacillus reuteri* had the highest percentage hydrophobicity of 19.79±0.05 while *Lactobacillus fermentum,* has the lowest

percentage of hydrophobicity of 9.94±0.01 as seen in table 7. Generally, the cell-adhesion of LAB to intestinal tract is the prerequisite for bacterial colonization and proliferation. The adhesion of Lactic Acid Bacterial to surface of intestinal epithelial cells has been known to depend on bacteria concentration, interaction time and hydrophobicity (Sharma, & Kanwar, 2017). In this study, the distribution of hydrophobicity of LAB isolates from oral samples of respondents from the University of Ibadan were analyzed systematically. From this observation, the lower their hydrophobicity, the lower the attachment capability vice visa. This agrees with the findings of Whitley *et al.* (2015) and García-Cayuela et *al.* (2014) who both reported that low hydrophobicity value of LAB isolates could affect the distribution of LAB in the intestinal tract, which is a prerequisite for bacterial colonization and proliferation.

Table 7.

4. CONCLUSION

From this study, it can be deduced that Lactic Acid Bacteria isolated from oral sample showed a wide range of positive responses to various growth parameters to which they were subjected. They are acid tolerant, bile tolerant and hydrophobic, factors that encourage their ability to survive in the intestine, which in turn confers probiotic effect on human health. They are mesophilic organisms which produced bacteriocin – like antibacterial secondary metabolites, a factor which helps the organisms to be used as a bio-preservatives industrially in the food industries to inhibit the growth of food spoilage pathogen.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1a. Biochemical characterization of LAB isolates from oral samples of respondent from different sections of the University of Ibadan.

Isolate code	Gram reaction	Cell morphology	Motility	Catalase	Oxidase	Indole	Starch hydrolysis	Methyl red	Voges proskeur	Nitrate reduction	Endospore former
A2	+	Cocci	-	-	-	-	-	+	-	-	-
A3	+	Cocci	-	-	-	-	-	+	-	-	-
A4	+	Cocci	-	-	-	-	-	+	-	-	-
A6	+	Rod	-	-	-	-	-	+	-	-	-
A7	+	Rod	-	-	-	-	-	+	-	-	-
A8	+	Rod	-	-	-	-	-	+	-	-	-
A9	+	Rod	-	-	-	-	-	+	-	-	-
A10	+	Rod	-	-	-	-	-	+	-	-	-
A11	+	Rod	-	-	-	-	-	+	-	-	-

Isolate code	**Homo/Het Ferment**	**Growth at 15°C**	**Growth at 30°C**	**Growth at 37°C**	**Growth at 41°C**	**Growth at 4% NaCl**	**H_2S Production**	**NH_3 from Arginine**
A2	HET	-	+	+	+	-	-	+
A3	HET	-	+	+	+	+	-	+

- =Negative, + = Positve,

Table 1b. Biochemical characterization of Lactic Acid Bacteria isolates from oral samples of respondent from different sections of the University of Ibadan

A4	HET	+	+	+	+	+	-	+
A6	HET	-	+	+	+	+	-	+
A7	HM	+	+	+	+	+	-	+
A8	HET	+	+	+	+	+	-	+
A9	HET	+	+	+	+	+	-	+
A10	HET	+	+	+	+	+	-	+
A11	HET	+	+	+	+	+	-	+

- =Negative, + = Positve,
- HET= Heterofermenter, HM= Homofermenter

Table 2. Molecular identification and accession numbers of the Lactic Acid Bacteria isolated from oral samples of respondents from different areas of the University Of Ibadan.

Sample ID	Scientific Name	Maximum Score	Total Score	Query Cover	E value	Percentage Identification	Acc. Len	Accession number
A2	*Pediococcus acidilactici*	2689	2689	99%	0	99.86%	1468	OM763689
A3	*Leuconostoc lactis*	2636	2636	99%	0	99.86%	1438	OM763690
A4	*Leuconostoc mesenteroides*	2669	2669	100%	0	100.00%	1445	OM763691
A6	*Lactobacillus acidophilus*	2647	2647	99%	0	99.79%	1444	OM763692
A7	*Lactobacillus reuteri*	2579	2579	94%	0	100.00%	1472	OM763693
A8	*Lactobacillus brevis*	2643	2643	100%	0	99.25%	1460	OM763694
A9	*Lactobacillus fermentum*	2710	2710	100%	0	99.93%	1470	OM763695
A10	*Lactobacillus plantarum*	2684	2684	100%	0	99.93%	1456	OM763696
A11	*Lactobacillus casei*	2671	2671	99%	0	99.79%	1464	OM763697

Table 3. Growth of lactic Acid Bacteria from oral samples of respondent from different sections of the University of Ibadan at 4% concentration of Nacl, $MgSO_4$, KNO_3, $NaNO_3$ and $MnSO_4$.

Isolate Code×	Nacl	$MgSO_4$	KNO_3	$NaNO_3$	$MnSO_4$
A2	0.687±0.01	0.750±0.01	0.866±0.02	0.519±0.02	0.345±0.01
A4	0.857±0.01	0.830±0.03	0.904±0.01	0.690±0.01	0.526±0.01
A3	0.955±0.02	1.030±0.05	1.053±0.02	0.716±0.04	0.421±0.01
A6	0.999±0.01	1.021±0.01	0.996±0.02	0.446±0.05	0.376±0.01
A7	0.895±0.02	0.996±0.03	0.876±0.01	0.324±0.02	0.664±0.03
A8	1.126±0.03	0.867±0.01	0.897±0.02	0.571±0.-01	0.721±0.05
A9	0.999±0.01	0.894±0.01	0.954±00.01	0.814±0.02	0.811±0.04
A11	1.201±0.01	1.196±0.03	1.206±0.01	1.204±0.03	0.652±0.01

A10	0.921±0.02	0.891±0.01	0.921±0.02	0.572±0.02	0.462±0.01
Control	0.152±0.01	0.152±0.01	0.152±0.01	0.152±0.01	0.152±0.01

Each value is a mean of three determinants and their standard error

Table 4. Growth of lactic Acid Bacteria from oral samples of respondent from different sections of the University of Ibadan at different acidic ranges

	pH/Growth (O.D.)			
Isolate code ×	**2**	**3**	**4**	**5**
A2	0.725±0.01	0.781±0.04	0.721±0.03	0.578±0.05
A4	0.610±0.05	0.652±0.01	0.712±0.02	0.610±0.03
A3	0.572±0.03	0.721±0.03	0.732±0.04	0.629±0.04
A6	0.801±0.01	0.798±0.02	0.625±0.05	0.627±0.03
A7	0.820±0.01	0.835±0.05	0.710±0.02	0.620±0.02
A8	0.851±0.04	0.862±0.03	0.625±0.06	0.651±0.05
A9	0.721±0.01	0.891±0.01	0.815±0.03	0.722±0.03
A11	0.913±0.04	0.827±0.01	0.725±0.01	0.689±0.04
A10	0.792±0.01	0.762±0.02	0.692±0.01	0.501±0.01

Each value is a mean of three determinant and their standard error

Table 5. Antagonistic activity of crude forms of Lactic Acid Bacteria supernatants against some selected entero-pathogens / indicator organisms.

Isolate code×	**Indicator Organisms / Activity (mm)(mean±SE)**			
	Escherichia coli	***Pneumonia aeruginosa***	***Staphylococcus. aureus***	***Bacillus. cereus***
A2	12.0±0.01	9.0±0.21	14.3±1.45	12.0±0.3

A4	10.5±0.08	12.0±0.01	13.7±0.20	9.7±0.2
A3	11.8±0.07	13.0±0.06	12.5±0.03	11.0±0.5
A6	13.3±0.33	11.2±0.73	15. 5±0.06	12.0 ±0.6
A7	13±0.08	13.0±0.04	16.8±0.42	12±0.8
A8	12.5±0.03	12.2±0.17	14.2±0.04	13.0±0.0
A9	12.3±0.05	13.2±0.44	13.7±0.02	12.0 ±0.4
A11	13.5±0.06	14.0±0.58	13.3±0.06	13.0 ±0.6
A10	15.0±0.0	11.3±0.44	11.3±0.03	13.0±0.4

Each value is a mean of three determinants and their standard error.

Table 6. Bile tolerance test of the Lactic Acid Bacteria isolated from oral samples of respondents from different areas of the University Of Ibadan.

Isolate code ×	Time (hrs)/Growth (OD)		Bile Tolerance
	With bile salt at 6hrs	Without bile salt at 6hrs	
A2	0.720±0.03	0.880±0.03	0.16
A3	0.898±0.01	0.851±0.05	- 0.047
A4	0.703±0.05	0.815±0.06	0.112
A6	0.759±0.02	0.905±0.02	0.146
A7	0.901±0.03	0.967±0.01	0.066
A8	0.802±0.04	0.905±0.02	0.103
A9	0.825±0.04	0.826±0.04	0.001
A10	0.834±0.03	0.925±0.05	0.088
A11	0.760±0.05	0.912±0.04	0.152

Table 7. Hydrophobicity measurement of the Lactic Acid Bacteria isolated from oral samples of respondents from different areas of the University Of Ibadan.

Isolate Code	H%(Mean ±SE)
A2	18.72±0.10
A4	16.04±0.07
A3	13.37±0.02
A6	17.12±0.07
A7	19.79±0.05
A8	12.42±0.01

A9	9.94±0.01
A11	17.98±0.09
A10	9.95 ±0.02

Each value is a mean of three determinants and their standard error.

H% - surface hydrophobicity.

- A2- *Pedicoccus acidilactici,* A3- *Leuconostoc lactis,* A4 - *Leuconostoc mesenteriodes,* A6 - *Lactobacillus acidophilus,* A7- *Lactobacillus reuteri,* A8 - *Lactobacillus brevis,*
- A9- *Lactobacillus fermentum,* A10- *Lactobacillus plantarium,* A11- *Lactobacillus casei,*

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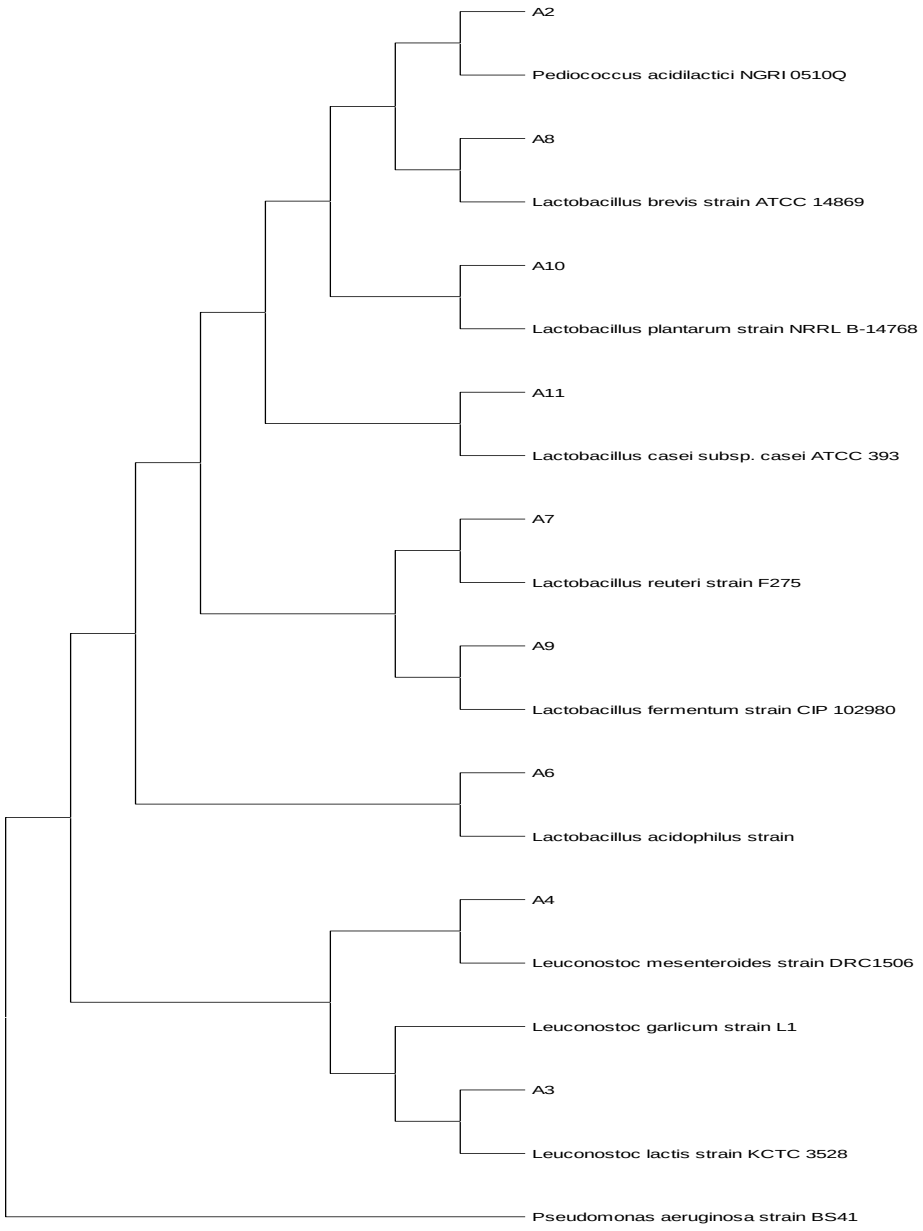

Figure 1. Phylogenetic relationship between the Lactic Acid Bacteria