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Research Article

Antibacterial Potentials of *Lactobacillus plantarum* from Fermented Food Origin as Probiotic Candidate in the Treatment of African Catfish (*Clarias gariepinus*) Bacterial Diseases

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ABSTRACT

Over the years, research on lactic acid bacteria isolated from fish gut against fish gram negative and fish gram positive pathogenic bacteria have been carried out. In this study, lactic acid bacteria of fermented food origin ("wara and fufu") were examined for their probiotic potentials against fish bacterial pathogens. Samples from fermented cow milk (wara) and fermented cassava (fufu) were stored in ice as: SW6, SW5, SW4, SF4, SF3, and SF2. Serial dilution was carried out with a 10-fold serial dilution. De Man, Rogosa and Sharpe (MRS) agar and de Man, Rogosa and Sharpe (MRS) broth were prepared and used for bacterial growth. Physiological, biochemical charactersations such as gram staining, bile salt tolerance, antibiotics susceptibility test and molecular characterisation of Lactic acid bacteria were carried out. The results revealed that the isolates were gram-positive, catalase negative, cocci, non-haemolytic, and non-pathogenic. The inhibition zones observed were: SW6 (24.7± 0.6) mm against *Aeromomas hydrophila*, SF4 produced inhibition zone of (16.3±0.6) mm against *Aeromomas hydrophila*, inhibition zone of (18.5±0.7) mm was produced in SF3 against Bacillus subtilis. All the isolates were susceptible to Chloramphenicol, Ampicillin, Clindamycin and Erythromycin. Molecular characterisation of isolates with high inhibition zones using 16S ribosomal RNA gene partial sequence identified two probiotic strains as SW6 and SF4. These strains were 99% identical to Lactobacillus plantarum strains R762 and AT4. It was deduced from this study that, for the purpose of bio-conservation, probiotics isolated from fermented food origin could replace fish-gut probiotics in treatment of fish bacterial diseases.

Keywords: *Lactic acid bacteria, antagonistic activity, Alternative treatment, Fish pathogens*

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INTRODUCTION

Aquaculture has been known as one of the most rapidly growing food producing industries (Otubusin *et al*., 2009). Antibiotics use has receded over the years due to its environmental hazards in the ecosystems (Sotolu *et al*., 2009). Antibiotics though help in eradicating pathogenic microbiota from culture species, they consequently eliminate beneficial bacteria from such culture species (Hoseinifar *et al*., 2016, 2017b). Due to this danger, findings are going-on worldwide for replacement of antibiotics with safer substitutes in aquaculture in controlling pathogenic organisms that cause diseases (Sayes *et al*., 2018). Research is on-going on the use of probiotics as substitutes to antibiotics in fish bacterial disease treatment. Parker (1974) defined probiotics as living biological entity that alter the intestinal microbes. Probiotics include Gram-positive, Gram-negative bacteria, yeasts, bacteriophage, and single-celled algae (Llewellyn *et al*., 2014). In aquaculture, it is necessary to ensure the selection of a non-toxic strain of probiotics on a target host to avoid antibacterial toxin secretion (Van Doan *et al*., 2017). Hence this study evaluated the antibacterial potentials of *Lactobacillus plantarum* from fermented food origin as probiotic candidate in the treatment of African catfish (*Clarias gariepinus*) bacterial diseases.

In order to explore Antibacterial potentials of *Lactobacillus plantarum* from fermented food origin as probiotic candidate in the treatment of African catfish (*Clarias gariepinus*) bacterial diseases, the present work was designed to investigate the biochemical, physiological and molecular characterisations of probiotics isolated from fermented food origin as probiotic candidate in the treatment of African catfish (*Clarias gariepinus*) bacterial diseases.

MATERIALS AND METHODS

Isolation of probiotic candidates: Specimens from zymotic cow milk (wara) and zymotic cassava (fufu) were collected and stored in ice. Homogenised specimens of these products were diluted serially by using 10-fold serial dilution in peptone water. De Man, Rogosa and Sharpe (MRS) agar and de Man, Rogosa and Sharpe (MRS) broth were prepared and utilized for bacterial proliferation according to manufacturer's recommendations. These were incubated under anaerobic condition for 24-48 hours at 30ºC, pH of culture solution was reconstituted by the addition of 0.1N NaOH and 0.1N HCl.

Territories making-up visible zones on the MRS agar plates were picked out and identification of lactic acid bacteria was presumptively carried out by physiological and biochemical Charactersations. These included Gram staining, catalase and oxidase production, cell morphology, colonial characteristics, pH determination, antibiotics susceptibility test and bile salt tolerance. Molecular characterisation was carried out to identify lactic acid isolates. Pure cultures of isolates re-traced on MRS agar medium were collected. The culture was maintained in MRS agar and MRS broth and stored at 4ºC. These were activated on MRS agar for 24 hours before experimental use (Iranmanesh *et al.*, 2014).

Biochemical and Physiological Characterisations

Cell morphology: Gram staining was carried out and cell forms were viewed under microscope. Cells morphology observed were Gram positive with rod-like or cocci cluster (Iranmanesh *et al*., 2014).

Catalase test: This was carried out by addition of hydrogen peroxide (H2O2) 3% to inoculums for the production of catalase enzyme (Todorov *et al*., 2011).

Bile Tolerance: Strains of isolates developed in De Man, Rogosa and Sharpe (MRS) broth which contained 0.2, 0.4 and 0.6% of bile. Aseptic levelled-bottom 96-well microtitre containers were used for the assay.180 µl of the medium was used to fill-in each of the wells in the microtitre plates and 20 µl of the cultures obtained in MRS broth (OD660nm = 0.2) at 37ºC was introduced into it. At every 12hours the optical density readings were taken at 660 nm while cultures developed in MRS broth containing 0% bile were used as a standard. (Todorov *et al*., 2011).

pH tolerance: Strains developed in MRS broth were adjusted to pH of 2, 2.5, 3.0, 4.0, and 7.0. Aseptic levelled-bottom 96-well microtitre containers were used for the assay.180 µl of the medium was used to fill-in each of the wells in the microtitre plates and 20 µl of the cultures obtained in MRS broth (OD540nm = 0.2) at 37°C was added to it. At mid-day, the optical density figures were taken at 540 nm while cultures developed in MRS broth at pH 7.0 were the standard (Todorov *et al.*, 2011).

Heamolysis Test: Heamolysis was observed by re-streaking isolates on a 24-hour culture on MRS agar which had an inclusion of 5% human blood (Mourad and Eddine, 2006).

Antibiotic sensitivity test: The antibiotics discs used for susceptibility assay were vancomycin, ciprofloxacin, penincillin, chlorampenicol, erythromycin, tetracycline, metronidazole, clidamycin, imipenim and ampicillin. Lactic acid bacteria was swabbed on agar containers and supplied with heat at 37ºC for one day with antibiotic discs. Zones of inhibitions were measured afterwards (Bauer *et al.*, 1966).

Antibacterial activity of lactic acid bacteria (LAB): The antibacterial activity of *Lactobacillus plantarum* was carried out by centrifuging *Lactobacillus plantarum* which had been grown on a 24-hour de Mann Rogosa Sharpe (MRS) broth according to the manufacturer's specifications. Precisely 5mL of the metabolites of selected LAB against the pathogens were done using eight pathogenic organisms that were biochemically characterised. They were *Aeromonas hydrophila, Klebsiella, pneumonia, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Streptococcus agalacticeae* (Todorov and Dicks, 2006). These pathogens were got from the Department of Microbiology of the University of Ibadan. All microbes were kept on nutrient slants. Afterwards, these pathogenic organisms were sub-cultured into freshly prepared nutrient broth (Simova *et al.*, 2009). Nutrient agar was used all through as the medium for growth of the pathogens.

All media were prepared according to the manufacturers' manuals. The medium was weighed and dissolved in measured distilled water in a conical flask, capped tightly and then sterilized at 121ºC for 15 minutes by autoclaving. It was then allowed to cool to 40ºC, and 20 mL of it was poured to aseptic petri plates and allowed to harden. Well diffusion technique was used to screen *Lactobacillus plantarum* for antimicrobial activity (Simova *et al.*, 2009). The medium was seeded with the test microbes by employing sterile swab sticks in spreading the inoculums of each microbe evenly over the surface of the prepared plates. After the seeded plates were dried, standard cork borer of 5mm in diameter was used to make holes on the surface of prepared plates. Lactic acid bacteria were introduced into the holes using sterile syringes of 2 5mL. The containers were all exposed to heat aerobically at 37ºC for one day. Thereafter, zones of inhibition of the growths were observed and measurements of the zones were taken using a transparent ruler (Sezer and Güven, 2009).

Molecular characterisation: DNA was extracted using the protocol stated by Saraniya and Jeevaratnam (2012). Distinct territories developed on medium were moved to 1.5 mL of liquid medium and cultures developed on a shaker for two (2) days at 28ºC. Afterwards, cultures were shaken at 4600x g for 5 minutes. The resultant rounded balls were re-suspended in 520 µL of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). There were additions of fifteen microlitres of 20% SDS and 3 µL of Proteinase K (20 mg/ml). The mixture was exposed to heat for 60 minutes at 37ºC, thereafter 100 µL of 5 M NaCl and 80 µL of a 10% CTAB concentration in 0.7 M NaCl were added and mixed. The suspension was exposed to heat for 10 minutes at 65ºC and placed on ice for 15 minutes (Saraniya and Jeevaratnam, 2012). There was an inclusion of the same

quantity of chloroform: isoamyl alcohol (24:1), this was proceeded by incubation on ice for 5 minutes and centrifugation at 7200 x g for 20 minutes. The aqueous period was moved to another tube, isopropanol (1:0.6) was included and DNA was precipitated at –20ºC for 16 h. DNA was retrieved by centrifugation at 7200 x g for 10 mins, rinsed with 500 µL of 70% alcohol, air-dried at room temperature for about three hours dissolved in 50 µL of TE buffer (Agaliya and Jeevaratnam, 2013).

PCR reaction cocktail: 10 µL of 5x GoTaq neutral reaction, 3 µL of MgCl2, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F 5′- AGA GTT TGA TCM TGG CTC AG-3′ and - 1525R, 5′AAGGAGGTGATCCAGCC-3′. Primers and 0.3units of Taq DNA polymerase (Promega, 1 USA) added up to 42 µl with purified distilled water 8µl DNA template. PCR carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Incorporated USA) PCR profile a first change of nature, 94ºC for 5 min; 30 cycles, of 94ºC for 30 seconds, 50ºC for 60 seconds and 72ºC for 1 minute 30 seconds; and a last prolongation at 72ºC for 10 minutes and cooled at 4ºC gel (Agaliya and Jeevaratnam, 2013).
An even quantity chloroform: isoamyl alcohol (24:1) was included, it was proceeded by incubation on ice for 5 minutes and centrifugation at 7200 x g for 20 minutes. The aqueous phase was transferred to a new tube, isopropanol (1:0.6) was added and DNA was precipitated at –20ºC for 16 h. DNA was got by centrifugation at 7200 x g for 10 min, rinsed in 500 µL of 10 70% ethanol, air-dried at room temperature for about 180 minutes and finally dispersed in 50 µL of TE buffer (Agaliya and Jeevaratnam, 2013).

Integrity: The integrity of the amplified 1.5Mb gene fragment was checked on a 1% Agarose gel ran to confirm amplification. This was carried out through mixing 8µl of amplified product to 4µl of loading dye and ran on the hardened Agarose gel at 110V for about 60 mins. Picture was taken under UV light. Also the amplified product was checked on a nano drop of model 2000 from thermo 18 scientific to ascertain the quantity of the concentration of the amplified product (Agaliya and Jeevaratnam, 2013).

Purification of amplified product: After gel integrity, PCR reagents were eradicated with the use of amplified fragments which were ethanol purified. In few minutes, 7.6 µL of Na acetate 3M and 240 µL of 95% ethanol were introduced to each about 40µL PCR amplified product in a distinct sterile 1.5 µL tube eppendorf, mixed vigorously by vortexing and positioned at -20ºC for at least 30 min. Centrifugation for 10 minutes at 13000 g and 1 4ºC proceeded by supernatanat eradication (invert tube on trash once) thereafter the small balls were cleaned by adding 150 µL of 70% ethanol and mixed then centrifuged for 15 minutes at 7500 g and 3 4ºC. All supernatants were displaced (inverted tube on trash) and the tubes were turned upside down on thin film and dried in a cupboard at room temperature for 10-15 minutes. This was re-suspended with 20 µL of purified distilled water and kept in -20ºC before sequencing. The purified fragment was examined on a 1.5% Agarose gel ran on a voltage of 110V for approximately 60 mins as before to ascertain the appearance of the purified product (Hata and Ohmomo, 2010).

Sequencing: The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems with the producer's pamphlet while the sequencing kit employed was that of Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 employed for all genetic results. (Hata and Ohmomo, 2010).
Genetic results were obtained through Bio-edit software and MEGA 6. This was initially employed in producing whole sequenced nucleotide sequence of individual bacteria isolate. The sequence result got employing the 1525R reverse primer was turned upside down, completed using bio-edit software and systematically arranged with sequence data of 27F. This was proceeded by bringing into existence of a general sequence to get the whole sequence of the bacteria 16S product. This general Nucleotide sequence were examined to observe similarities and differences with already known sequences on the data base by the Basic Local Alignment Search Tool (BLAST) network services of the National Centre for Biotechnology Information (NCBI) database. Bacteria species were named according to the highest likeness with the best aligned reference sequence of NCBI database. The general sequences of the total bacterium isolates with various strains already known in the NCBI *Lactobacillus plantarum* strain CWBI B-76, *Lactobacillus plantarum* strain AT4, *Lactobacillus plantarum* strain R762, *Lactobacillus fermentum* strainBCS27, *Lactobacillus fermentum* 16S gene strain: JCM 8581, *Lactobacillus fermentum* strain KKL3, *Lactobacillus fermentum* strain F4S8 and (*Lactobacillus brevis* strain RO97) were brought together using Bio-edit then multiple sequence alignment was carried out and transferred to MEGA 6 for phylogenetic analysis. A dendrogram was built by employing the highest likelihood tree with 1000 bootstrap copies built on Single Nucleotide Polymorphisms (SNPs), additions/subtractions (INDELS), and/or length variants in the 16S rDNA regions.

RESULTS

Biochemical characterisation of lactic acid bacteria isolates: Absorbance of fermented lactic acid bacteria isolates is shown in Table 1.

Table 1:
Absorbance of fermented lactic acid bacteria isolates at 540nm using spectrophotometre

Isolates	P^H at 7.0	P^H at2.0	P^H at3.0	P^H at4.0
Sw6	6.5	0.7	0.1	0.1
Sw5	6.3	0.1	0.1	0.0
Sw4	8.9	0.1	0.1	0.0
Sf4	7.0	0.2	0.1	0.0
Sf3	3.7	0.2	0.1	0.0
Sf2	3.7	0.5	0.1	0.1

Footnote:
Sw5, Sw6, Sw4= Strains of lactic acid bacteria isolated from "wara" (fermented cow milk)
Sf4, Sf3, Sf2=Strains of lactic acid bacteria isolated from "fufu" (fermented cassava)

It was observed that at heightened acidic conditions, the growth of the isolates was lowered. The bile salt tolerance of fermented lactic acid bacteria isolates is shown in Table 2. It was observed that as the concentration of the bile salts increased, the growth rates of the isolates decreased at the different concentrations. The pure cultures were gram positive, catalase negative, rod-like and cocci and they appeared in clusters. They were all unable to produce haemolytic zones, which implied that they were confirmed to be non-pathogenic. These indicated the viability of the isolates to 17 be used as probiotic candidate.

Table 2:
Bile salt tolerance of fermented probiotic isolates at 540nm using spectrophotometre

Isolates	Spec at 2.0	Spec at 2.5	Spec at 4.0
Sw6	0.2	0.2	0.1
Sw5	0.3	0.3	0.2
Sw4	0.3	0.1	0.0
Sf4	0.4	0.2	0.1
Sf3	0.3	0.3	0.1
Sf2	0.3	0.2	0.0

Footnote:
Sw5, Sw6, Sw4= Strains of Probiotics isolated from "wara" (fermented cow milk)
Sf4, Sf3, Sf2=Strains of Probiotics isolated from "fufu" (fermented cassava

Antagonistic activities of lactic acid bacteria separated from zymotic food products against selected fish pathogens: The antibacterial activity of lactic acid bacteria pure cultures against selected fish pathogens is shown in Table 3. There were appreciable zones of inhibition of pure cultures tested against streptomycin.

On the contrary, no visible antibacterial activity was produced with the sterile water against tested pure cultures. From the results on the antibacterial activity of the tested isolates, it was observed that Sw6 had the most obvious zone of inhibition zone of (24.7±0.6) mm against *Aeromonas hydrophila*. It was observed that Sf4 produced zone of inhibition of (16.3±0.6) mm against *Aeromonas hydrophila.* The Sw5 and Sw4 produced no zones of inhibition against Streptococcus *agalacticeae*. Sw6 and Sf4 produced no zones of inhibition against *Klebsiella pneumonia.* Among the isolates from fufu Sf3 produced the most obvious zone of inhibition against *Bacillus subtilis* (18.5±0.7) mm. Sf2 produced no zones of inhibition against *Pseudomonas aeruginosa.*

Antibiotic susceptibility test of lactic acid bacteria isolated from zymotic food products against selected antibiotics (disc size=5mm): The antibiotic susceptibility tests of fermented lactic acid bacteria isolates is shown in Table 4. It was observed that all the pure cultures resisted imipenim, ciprofloxacin and vancomycin. Pure culture from "wara" (Sw5) was resistant to ampicillin and penincillin, while those from "fufu" (Sf3 and Sf2) resisted metronidazole. However, all the strains isolated from "wara" (SW4, SW5, and SW6) and "fufu" (SF2, SF3, SF4) were susceptible to tetracycline, chloramphenicol, ampicillin, clidamycin and erythromycin.

Table 3
Antagonistic Activities of Lactic Acid Bacteria isolated from fermented food products against selected fish Pathogens (mm)

Pathogens	Probiotics Isolates							
	Sw6	Sw5	Sw4	Sf4	Sf3	Sf2	+ve	-ve
Aeromonas hydrophila	24.7±0.6^{a}	17.3±0.6^{c}	19.3±0.6^{b}	16.3±0.6^{c}	13.3±0.6^{d}	N. A.	N. A.	11.33±1.2^{e}
Klebsiella pneumonia	N. A.	20.7±1.2^{a}	16.3±0.6^{b}	N. A.	14.3±0.6^{c}	15.0±0.4^{c}	N. A.	13.7±0.6^{d}
Bacillus subtilis	13.5±0.7^{c}	14.5±0.7^{c}	17.5±0.7^{b}	17.7±0.6^{b}	18.5±0.7^{a}	10.5±0.7^{d}	N. A.	11.0±1.4^{d}
Staphylococ-cus aureus	4.7±8.1^{e}	19.0±4.4^{a}	14.7±0.6^{c}	5.0±8.7^{e}	13.3±4.2^{d}	16.7±3.0^{c}	N. A.	17.3±6.4^{b}
Escherichia coli	10.0±8.7^{d}	7.3±12.7^{e}	14.3±0.6^{b}	10.0±8.7^{d}	12.0±2.0^{c}	5.7±9.8^{f}	N. A.	19.0±1.7^{a}
Proteus vulgaris	12.7±3.1^{b}	6.7±5.8^{e}	10.3±3.2^{c}	8.0±5.3^{d}	4.0±6.9^{f}	6.0±5.3^{e}	N. A.	15.7±2.1^{a}
Pseudomon-as aeruginosa	11.3±1.2^{c}	4.0±6.9^{d}	2.7±4.6^{e}	12.3±7.2^{b}	N. A.	N. A.	N. A.	19.0±4.6^{a}
Streptococc-us agalacticeae	18.0±5.3^{b}	N. A.	N. A.	15.3±1.2^{c}	10.3±8.1^{e}	11.7±10.1^{d}	N .A.	21.0±1.0^{a}

Means with the same letter within row are not significantly different (P>0.05)

Table 4:
Antibiotic susceptibility test of lactic acid bacteria isolated from fermented food products against selected antibiotics (disc size=5mm)

	Zones of Inhibition									
Isolates	Amp	P	MTZ	VA	C	TE	CIP	DA	IPM	E
Sw6	5	16	3	R	23	9	R	25	R	19
Sw5	R	R	R	R	24	17	R	27	R	23
Sw4	4	7	7	R	23	11	R	29	R	24
Sf4	6	17	5	R	24	12	R	25	R	22
Sf3	6	4	R	R	20	13	R	24	R	20
Sf2	9	8	R	R	22	22	R	27	R	25

Footnote: Amp- Ampicillin, P- Penincilin, MTZ- Metronidazole, VA- Vancomycin,C- Chloramphenicol, TE- Tetracycline, DA- Clidamycin, IPM- Imipenim, E- Erythromycin R- Resistant, Sw5, Sw6, Sw4=Strains of lactic acid bacteria isolated from "wara" (Fermented cow milk), Sf4, Sf3, Sf2=Strains of lactic acid bacteria isolated from "fufu" (fermented cassava).

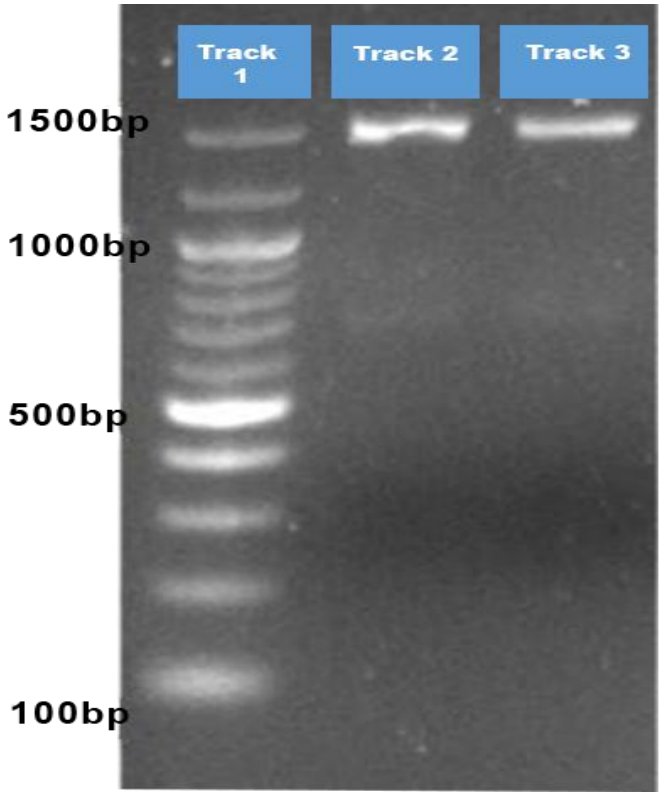

Plate 1:
Agarose gel showing the amplification of 16Sr DNA of*Lactobacillus plantarum* strains
Track 1 contains the 100 base pair of 16Sr DNA ladder
Track 2 contains 16Sr DNA (1500 base pair) of *Lactobacillus plantarum* strain AT4
Track 3 contains 16Sr DNA (1500 base pair) of *Lactobacillus plantarum* strain R762

Phylogenetic Tree and Gene sequence of identified *Lactobacillus plantarum* strains: From the molecular characterisation using the 16S ribosomal RNA gene partial sequence, two isolated strains of the pure cultures were molecularly identified after selection from the biochemical and morphological screening. They were SW6 (from fermented cow milk "wara") was 99% identical to *Lactobacillus plantarum* strain R762 while SF4 (from fermented cassava "fufu") was 99% identical to *Lactobacillus plantarum* strain AT4.

Phylogenetic analyses: From the molecular characterisation using the 16S ribosomal RNA gene partial sequence, two isolated strains of the pure cultures were molecularly characterised after selection from the biochemical and morphological screening.

The phylogenetic analysis based on multiple sequence alignment of the bacteria pure isolates tested here, along with GenBank reference sequences is shown in Figure 1. The major knob of the tree differentiated the total species with high bootstrap values. Reference sequences of the three species *Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus fermentum* used in the phylogeny computation were clearly delineated into 2 major phylogenetic clades with the species of *Lactobacillus fermentum* grouped separately from those of *Lactobacillus brevis* and *Lactobacillus plantarum* Based on the phynogram, both sample W6 and F4 despite the SNPs and Indels were most identical to *Lactobacillus plantarum* strain R762, however, SW6 was closely related to *Lactobacillus plantarum* strain R762 compared to SF4. A clearer confirmation of the sample identity was determined using the NCBI database. Sample SF4 when blasted in NCBI was recorded to be 99% identical to *Lactobacillus plantarum* strain AT4 while SW6 was 99% identical to *Lactobacillus plantarum* strain R762. This indicated that both samples were *Lactobacillus plantarum*

DISCUSSION

In recent years, studies have been carried out on lactic acid bacteria got from fish gut against fish gram negative and fish gram positive pathogenic bacteria (Brunt *et al*., 2007). In this study, lactic acid bacteria of fermented food origin (wara and fufu) were examined. Probiotic properties which included morphological and biochemical tests were carried out on the probiotics isolates. Brunt *et al.* (2007) experimented on *Lactobacilli* isolated from fish gut and reported that *lactobacilli* isolated from fish gut were more potent in inhibiting fish pathogenic bacteria.

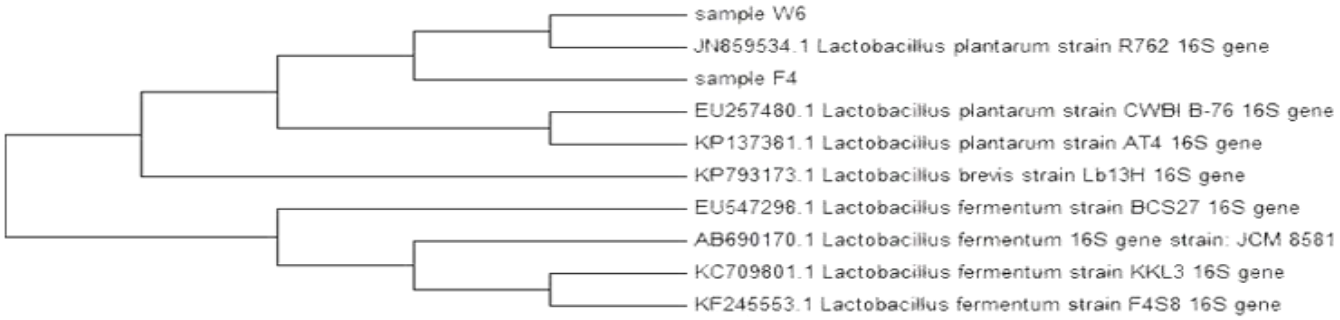

Figure 1:
Phylogenetic tree showing the isolates related to *Lactobacillus plantarum*
Lactobacillus plantarum SF4 was 99% identical to *Lactobacillus plantarum*strain AT4 (fermented cassava "fufu")
Lactobacillus plantarum SW6 was 99% identical to *Lactobacillus plantarum* strain R762 (fermented cow milk "wara").

For the purpose of bio-conservation of aquatic resources in the ecosystem, this experiment investigated the viability of substituting lactic acid bacteria from unconventional sources (food origin) with those from fish gut (conventional source) in the prophylactic and therapeutic treatment of fish bacteria diseases in-vitro.

From the results of this experiment, all the gram negative and gram-positive bacterial strains were inhibited by lactobacillus in-vitro. This was contrary to the reports of Gildberg *et al*. (1997), Adewoye and Lateef (2004) that Lactobacillus produced no zone of inhibition with gram positive and gram negative bacteria strains from C. gariepinus in-vitro. However, a corresponding result to this study was reported by Ogunshe *et al*. (2007) and Ogunshe (2008) that Lactobacilli obtained from Nigerian fermented foods produced high antibacterial inhibition against isolated fish bacterial pathogens and there was high antibiotic susceptibility of fish bacterial pathogens to tested antibiotics. This conformed to results of this experiment, which revealed a high susceptibility pattern of bacterial pathogens to tested antibiotics and a high inhibition of L. plantarum strains isolated from Nigerian fermented food against bacterial pathogens.

Molecular identification of lactic acid bacteria isolates

There is need for an accurate and definitive identification of micro-organisms. The 16SrDNA sequence based analysis is essential in understanding diversity of microbes and their strains identification within and across any group. There was at least a copy of highly conserved hyper-viable regions of 16SrDNA gene contained by in bacterial strains. Nevertheless, differences occur in the linear measurement and the sequence of 16 SrDNA ITS region. Hence, the 16 SrDNA ITS region is important in the characterisation of 16 bacterial species (Mohammed *et al*., 2011). The 16SrDNA gene is important due to the fact that the genome of all bacteria has in it the conserved gene. There is usually a distinctive difference in species identification due to a slight variability (Mohania *et al*., 2008).

Lactic acid bacteria are probiotic because they belong to the group of micro-organisms isolated from fermented food which are generally regarded as safe (GRAS). Its rapid recognition is an essential instrument in identifying various strains of probiotics and it helps to distinguish probiotics from other debilitating pathogens that may appear in zymotic foods. However the purpose of using 16SrDNA sequencing which is species-specific PCR reaction in this study was for proper identification of the two selected Lactic acid bacteria isolates. The genes grade was equal to the two pure cultures SW6 and SF4. Characterisation was carried out by the application of PCR activities for complete identification process. The obtained results had 99% reliability. This was considered reliable because PCR reactions are aimed at identifying specific genes of genera and species. Molecular identification of bacteria is anchored on the whole linear measurement of 16SrDNA gene sequence.

According to Paula *et al*. (2013) variances between genotypic and phenotypic tests are recognized for other bacteria including LAB. This tool is used to identify micro-organisms at sub-species level, especially for organisms that cannot be identified by other techniques. There is the tendency of poor reproducibility caused by differences of increase in size and structure of various organisms. This supports the study of Mohania *et al*., (2008) that explains that there were discrepancies in the results of biochemical tests of organisms due to lose of plasmids during culture.

The molecular method applied in this study ascertained the true identity of L. plantarum strains isolated from fermented food. One advantage of this method is that it can be reproduced at anytime and anywhere without alteration due to environmental variations (Adeyemo and Onilude, 2013). It can be recommended for proper identification of probiotics organisms from fermented foods. This method reduces time of analysis. It gives allowance for reproducibility of organisms that are delicate, stunted and of economic advantage (Shittu *et al*., 2006). The result of this experiment elucidates the above advantages and it agrees with that of Woo *et al*. (2008) who noted that there are LAB species which relate intimately with Lactobacillus species. On this premise, it is essential to properly identify LAB got from zygotic foods used as probiotics from those that are pathogenic such as *Leuconostic* sp. and *Pediococcus* sp.

In conclusion, the result of this study revealed six probiotic isolates which were cultured from fermented cow milk 17 'wara' and fermented cassava 'fufu'. They were: Sw4, Sw5, Sw6, Sf3, Sf4, and Sf5. The antibacterial 18 properties of the isolates revealed isolates Sw6 had the highest inhibition zone (24.7± 0.6) mm against 19 *Aeromonas hydrophila*. Among isolates from fermented cassava 'fufu' Sf3 produced the highest zone 20 of inhibition against Bacillus subtilis (18.5±0.7) mm. Sf4 produced the highest zone of inhibition 21 among the isolates from 'fufu' against Aeromonas hydrophila (16.3±0.6) mm. Hence, for the purpose of bio-conservation, probiotics from fermented food origin can be used for the treatment of fish bacterial diseases in place of the probiotics isolated from fish gut.

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