Original Research Article

Screening of microbilogical Isolates with Probiotic Potential of Tiger Nut Drink, Ogi and Palm Wine

ABSTRACT

Probiotics are live microorganisms that are very beneficial to human health when consumed in a sufficient amount. Screening and fingerprinting of isolates with probiotic potentials from indigenous food products were evaluated. Fresh palm wine, Ogi and Tiger nut drinks were bought from retailers in Obio-Akpor and Port Harcourt Local Government Area, Rivers State. These samples on getting to the lab in sterile containers were analysed using standard microbiological techniques for the enumeration and isolation of bacterial isolates. Identification of isolates relied on the biochemical and genomic techniques using standard methods. The probiotics were screened based on their ability to tolerate ethanol, bile salt, low pH, high salt concentration, lactose utilization and the production of biogenic amine. Antimicrobial susceptibility of the bacterial isolates (probiotics) was carried out using the Kirby-Bauer disc diffusion. Forty-two bacterial isolates which belonged to the genera: *Lactobacillus* sp, *Pediococcus* sp, *Enterococcus* sp and *Streptococcus* sp were identified. Genomic characterization of isolates showed that isolate NO2 has 83.4% pairwise identity with *Bacillus firmus* strain T1, Isolate NP2 has 86.5% pairwise identity with *Bacillus cereus* strain PKID1, NT8 has 80.3% pairwise identity with *Bacillus cereus* strain PV-G21. Results of screened probiotics showed that out of the forty-two bacterial isolates, only fifteen were non-spore producers and that they were tolerant to ethanol, low pH, NaCl and bile salt at all concentrations. Results of lactose utilization showed that only twelve out of the fifteen bacterial isolates utilized lactose. Results of biogenic amine production showed that only five out of fifteen bacterial isolates produced biogenic amine. The antibiotic susceptibility pattern of the screened bacterial isolates showed that they exhibited resistance to Pefloxacin, Gentamycin, Ampiclox, Amoxicillin, Rocephin, Ciprofloxacin; Streptomycin, Sceptrin and Erythromycin. They were highly resistant to Gentamycin and Zinnacef. *Bacillus firmus strain* T1, *Bacillus cereus* strain PKID1 and *Bacillus cereus* strain PV-G21 were identified as bacterial probiotics. Consumption of palm wine, Ogi and tiger nut drinks is highly recommended due to the availability of probiotics.

Keywords: microbiology, bacterial isolates, Genomic characterization, biogenic amine

INTRODUCTION

Probiotics are live microorganisms that can provide benefits to human health when administered in sufficient amounts that confer a beneficial health effect on the host (FAO/WHO, 2002). Alternatively, probiotics have been defined as live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989), as a viable mono or mixed culture of bacteria, which when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora (Huis and Havenaar, 1997).

Probiotics are poised as a valuable means of influencing the function of the gut ecosystem to improve nutritional status and health (Versalovic, 2013). Probiotics are poised as a valuable means of influencing the function of the gut ecosystem to improve nutritional status and health (Versalovic, 2013).

There is increasing evidence that probiotics are beneficial in the management of gastrointestinal disturbances such as diarrhoea, dysentery, and typhoid (Tambekar and Bhutada, 2010)., which in many cases their effects are mainly prophylactic in nature rather than therapeutic (preventive rather than curative) (Suskovic et al., 2001), and the major microorganisms used as probiotics belong to the LAB bifidobacteria and LAB Lactobacillus sp; the most commonly utilized group of microorganisms for their potential beneficiary properties as probiotics (Didari et al., 2014).

The use of probiotics to prevent and treat a wide variety of disease conditions has gained acceptance in the past decade (Britton and Versalovic, 2008). This is in fact, due to a need to find alternatives to traditional therapies such as the use of antibiotics as well as good treatments for gastrointestinal and other diseases. No probiotic strain provides all proposed health benefits, not even strains of the same species, and not all strains of the same species will be effective against defined healthy conditions (Figueroa-González et al., 2011).

Fresh palm wine is sweet, clear, neutral, and contains a small amount of protein, minerals (Opara et al., 2013), water, sugar, vitamins, and many flavour components. (Oyeku et al., 2009). Palm wine has several nutritional, medical, religious and social uses which have been reported to have increasingly enhanced the demand for this natural product (Faparusi, 1991; Odeyemi, 1977; Ikenebomeh and Omayuli, 1988; Uzogara et al., 1990; Iheonu, 2000). Biodiversity associated with palm wine is possible because it constitutes a good growth medium for numerous microorganisms especially for yeast, lactic acid and acetic acid bacteria (Bechem et al., 2007). Palm sap begins fermenting immediately after collection, due to natural yeasts in the air (often spurred by residual yeast left in the collecting container).

Ogi is a product of fermented maize (Zea mays) widely consumed in Africa (Amakoromo, 2011) and (Adams and Moss, 1995). It is popular in Nigeria and most parts of West Africa (Banigo and Muller,1972). Maize is rich in carbohydrates and minerals, including potassium and magnesium. It, however, contains trace amounts of amino acids mainly lysine and tryptophan, contributing to the low

content of protein, and trace amounts of vitamins, especially the B- vitamins (USDA, 2012). Lactic acid bacteria (*Lactobacillus plantarum* and *Streptococcus lactis*) and yeasts (*Saccharomyces cerevisiae*, *Rhodotorula* spp., *Candida mycoderma*, and *Debaromyces hansenii*) are predominantly involved in the fermentation of ogi, playing important roles as aroma development, microbial stability and flavour enhancement (Omemu and Faniran, 2011; Aworh, 2008).

Tigernut is known in Nigeria as Aya in Hausa, Ofio in Yoruba and Akiausa in Igbo and these varieties (black, brown and yellow) are cultivated (Umerie *et al*., 1997). Tigernut has long been recognized for its health benefits as they are rich in fiber, protein, natural sugars, minerals such as phosphorous and potassium and vitamins such as E and C. They have a high content of soluble glucose and oleic acid, along with high energy content (starch, fats, sugars and proteins (Mason, 2008).

Recently, there is awareness for increased utilization of tiger nuts (Belewu and Abodunrin, 2006; Belewu and Belewu, 2007). Being cultivated through continuance irrigation, tiger nut has to be properly dried before storage.

STATEMENT OF THE PROBLEM

The indigenous foods such as Tigernut, Palmwine and Ogi have been reported to harbor microorganisms with probiotic potentials (Ndikom and Elutade, 2016), yet many people still report stomach upset, as a result of consumption of these indigenous products despite the acclaimed presence of probiotic microorganisms.

AIM OF THE STUDY

This study is aimed at screening and obtaining fingerprints of isolates with probiotic potentials from indigenous food products.

MATERIALS AND METHODS

DESCRIPTION OF STUDY AREA

Aluu is located in the Niger Delta region; Rivers state. The area is situated between latitudes 4°56′2.06 north latitude and 6°56′37.36 east longitude.

Mile 3 market is located in Mile 3 Market, Nkpolu-Oroworukwo, Port Harcourt, Rivers State. It is situated between Latitude 4.824167 and Longitude 7.033611

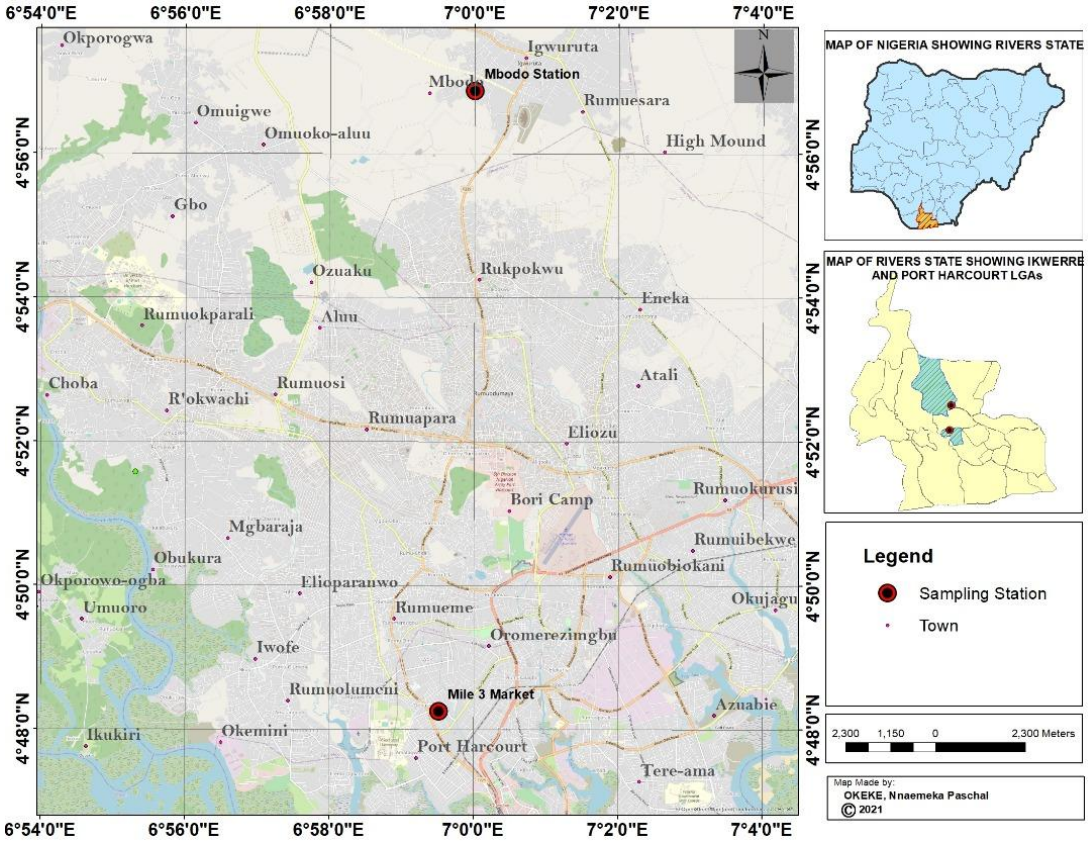

Image 1: Map of Study Area

COLLECTION OF SAMPLES

PALM WINE

Freshly tapped palm wine was collected at the point of tapping from traditional palm wine tappers in Aluu, Obio-Akpor, Rivers State, Nigeria. The samples were collected in sterile 1.5 litres sterile flasks and transported to the laboratory in coolers equipped with ice packs within one hour of tapping for analyses. This procedure is intended to keep the samples at about 2 - 4°C (Ibekwe et al. 2006; Chijioke and Ukaegbu- Obi 2016).

OGI

Ogi used in this study was bought from Mile 3 Market, Nkpolu-Oroworukwo, Port Harcourt, Rivers State. The sample was stored in a polyethene bag and transported to the Laboratory for analysis.

TIGERNUT DRINK

Tigernut drink used in this study was homemade to prevent contaminants. The sample was collected and transported to the laboratory in coolers equipped with ice packs within one hour of tapping in 1.5 litres sterile flasks for analyses. This procedure is intended to keep the samples at about 2 - 4°C (Ibekwe et al. 2006; Chijioke and Ukaegbu- Obi 2016).

SOURCE OF BACTERIA

Bacteria were isolated from three sources – Palmwine (an alcoholic beverage obtained from the fermentation of the sugary sap of various palm trees such as Raphia vinifera and Raphia hookeri) was purchased for Aluu, Rivers State, Nigeria. Tiger nut drink (a fermented drink obtained from tigernut *Cyperus esculentus lativum*), Ogi (a product of fermented maize, Zea mays was purchased from Nkpolu-Oroworukwo (Mile 3 market), Rivers State, Nigeria.

ISOLATION AND IDENTIFICATION OF BACTERIA

Ten (10)g of Ogi and Ten (10)ml of palm wine and Tigernut drink were homogenized with 90ml of 0.85% (w/v) normal saline and serially diluted in the same diluents. The bacteria were isolated by plating on MRS agar (mand demand-Rogosa-Sharpe agar) and incubated anaerobically at 30Oc for 72h using an anaerobic jar with gas-pak. The predominant bacteria were obtained from plates with the highest sample dilutions of 10-5. Initial characterization of isolates included colony morphology, gram staining and catalase test. Gram-positive, catalase-negative, oxidase negative were presumptively identified as LAB.

SCREENING

BILE SALT TOLERANCE

The medium with varying concentrations of bile salt (0.5, 1.0, 1.5 and 2.0%) were inoculated with each selected bacteria culture and incubated at 370c for 48 hrs. Then 0.1ml inoculums were transferred to MRS agar by pour plate method and incubated at 370c for 48hrs. the growth of LAB cultures on agar plates was used to designate isolates as bile tolerant (Tambekar and Bhutada, 2010).

ETHANOL TOLERANCE

Ethanol tolerance was tested in peptone water broth by adding 10ml of 10% and 15% (V/V) of ethanol into 100ml of MRS broth. The test organisms were each inoculated into this prepared media and incubated at 300c for 48 hrs. The growth of each test organism was measured by changes in the optical density (OD) before and after incubation using a standard curve (Sievers,2006).

pH TOLERANCE

In vitro assay to determine the resistance of isolates to theacidic conditions of the stomach is an essential attribute in probiotic selection. As the food remains in the stomach for at least 3 h (Thakkar et al., 2015). The isolated bacterial and fungal cultures were inoculated into sterile peptone water at a pH of 2 and incubated at 37℃ for 2-3days. The growth of each test organism was measured using a UV- Spectrophotometer (520UV) by observing the changes in the optical density (OD) before and after incubation using a standard curve. The wavelength of measurement was 550nm.

SCREENING FOR BIOGENIC AMINE PRODUCTION

The biogenic amine forming capacity of LAB isolates was determined qualitatively in a medium containing 20ml of 10% (w/v) of histidine, lysine, ornithine and tyrosine. Change in colour of the bromocresol purple used as an indicator to purple colour was considered as positive reactions to amino acid decarboxylase activity (Tamang and Tamang, 2009). Negative results may be obtained when the strain produces a low amount of amine that is not enough to cause the pH shift for colour change as mentioned by Bover-Cid and Holzapfel,2009.

LACTOSE UTILIZATION

The acid production by selected bacterial cultures was detected by observing the change in colour of the medium. Sterilized fermentation medium (10g peptone, NaCl 15g, phenol red 0.018g, lactose 5g, for 1L distilled water and final PH 7.0) was inoculated with different cultures and incubated at 350c for 24-48hrs. Positive: Visible turbidity in the broth, with or without a colour change from purple to yellow. Turbidity alone is indicative of a positive test. Negative: No turbidity and no colour change after 72 hours of incubation (Markov,2016).

GENOMIC IDENTIFICATION

The bacterial and fungal isolates which were screened for probiotics potentialities were further identified Molecularly using the Polymerase Chain Reaction (PCR). This was done by extracting the DNA, carrying out PCR and sequencing the amplified DNA (amplicon).

EXTRACTION (BOILING METHOD) OF THE DNA

Extraction was done using a ZR fungal/ bacterial DNA prep extraction kit. Aeavy growth of the pure culture of the suspected isolate was suspended in 200 microlitres of isotonic buffer into a ZR Bashing Bead Lysis tubes and 750 microlitres of lysis solution were added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tubes were centrifuged at 10,000 xg for 1 minute. Four hundred (400) microlitres of supernatant were transferred to a Zymo- Spin IV Filter (orange top) in a collection tube and centrifuged at 700xg for 1 minute. One thousand two hundred (1200) microlitres of fungal/ bacterial DNA binding buffer was added to the filtrate in the collection tubes bringing it to a final volume of 1600microlitre. Eight hundred (800) microlitres were then transferred to a Zymo – spin IIC column in a collection tube and centrifuged at 10,000xg for 1 minute and the flow-through was discarded from the collection tube. The remaining volume was transferred to the same Zymo- spin and spun. Two hundred (200) microlitre of the DNA pre- washed buffer was added to the Zymo- spin IIC in a new collection tube and spun at 10,000xg for 1 minute followed by the addition of 500 microlitres of fungal/ bacterial DNA wash buffer and centrifuged at 10,000xg for 1 minute. The Zymo- spin IIC column was transferred to a clean 1.5 microlitre centrifuge tube. 100 microlitre of DNA elution buffer was added to the column matrix and centrifuged at 10,000 xg microlitre for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degrees for other downstream reactions (Fedricks *et al*., 2005).

AMPLIFICATION OF 16S rRNA

The ITS region of the rRNA genes of the fungal isolates was amplified using the ITS4: 5'-TCCTCCGCTTATTGATATGS-3' and ITS5: 5'-GGAAGTAAAAGTCGTAACAAGG-3', while the 16S rRNA of the bacterial isolates were amplified using the 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA primers. All was done separately on ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: X2 Dream Taq Master mix (Taq polymerase, dNTPs, MgCl), the primers at a concentration of 0.4 M and the extracted DNA as a template. The PCR conditions were as follows: Initial denaturation at 950C for 5 minutes, denaturation at 950C for 30 seconds, annealing at 530C for 30 seconds, extension at 720C for 30 seconds for 35 cycles and final extension at 720C for 5 minutes. The product was resolved on 1% agarose gel at 120V for 15 minutes and visualized on a blue light transilluminator (Fedricks et al., 2005).

SEQUENCING

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer. The sequencing was done at a final volume of 10µl, the components included 0.25ul BigDye terminator v1.1/3.1, 2.25µl of 5 x BigDye sequencing buffer, 10uM primer, PCR primer and 2-10ng PCR template per 100bp. The sequencing condition was as follows: 32 cycles of 960C for 10s, 550C for 5s and 600C for 4 minutes (Agi et al., 2017).

ANTIMICROBIAL SUSCEPTIBILITY OF ISOLATES

Each identified bacterial isolate was subjected to antibiotic resistance profiling against 10 widely used antibiotics viz. The distinct antibiotics used for screening includes; Ofloxacin (30µg), Streptomycin (30µg), Septrin (30µg), Sparfloxacin (10µg), Chloramphenicol (30µg), Ciprofloxacin (10µg), Ampicillin (30µg) Augmentin (30µg), Gentamycin (10µg) and Pefloxacin (10µg). All assays were performed in Mueller-Hinton agar plates using the Kirby-Bauer disk diffusion method. Sterile swab sticks were dipped into test tubes containing 18-24hrs isolates prepared according to McFarland's standard and seeded horizontally and vertically unto the surface of freshly prepared Mueller-Hinton agar. The plates were prepared in triplicates and properly labelled. These were later incubated at 37 0C for 24 hours. After incubation, zone diameter was recorded and interpreted as susceptibility, resistance or intermediate (CLSI, 2017).

RESULTS AND DISCUSSIONS

A total of forty-two bacterial isolates was characterized from different indigenous food products such as palm wine, tiger nut drink and Ogi were subjected to a different screening test for their probiotic potentials. Out of the 42 bacterial isolates screened, fifteen isolates were non-spore formers and were suspected *Lactobacillus, Streptococcus* and *Pediococcus* sp based on their phenotypic characteristics. Also, the cultural, morphological, and biochemical characteristics of *Lactobacillus* spin this study showed resemblance with other reported probiotic strains of *L. plantarum*, which were isolated from a wide variety of fermented foods (Palachum *et al.*, 2018; Nwachukwu *et al*., 2019).

Results showing the characteristics and probable identities of the bacterial isolates are presented in Table 1 Results showed that the bacterial isolates belonged to three genera: Lactobacillus, Pediococcus and Streptococcus *sp.* The phenotypic characteristics as illustrated in the result showed that they were all oxidase negative, non-spore formers. Streptococcus sp and Lactobacillus *sp* were catalase-negative while Pediococcus *sp* had delayed catalase-positive reactions compared to the strong catalase reaction of Staphylococcus *sp* which was used as control.

Table 1: Morphology and Biochemical Characteristics of Bacterial Isolates

Isolate	Morphology	Shape	Gram	Catalase	Oxidase	Indole	MRVP	Spore stain	Lactose	Suspected Organism
N01	White, Irregular, Large	Rod	+	-	-	-	-	No spore	+	*Lactobacillus* sp
N02	Yellow, Irregular, Small	Rod	+	+	-	-	-	No spore	+	*Pediococcus* sp
N05	Creamy, Irregular, Large	Cocci	+	-	-	+	-	No spore	-	*Enterococcus* sp
N07	Creamy, Irregular, Large	Cocci	+	-	-	-	+	No spore	-	*Streptococcus* sp
NP2	White, Circular, Large	Rod	+	-	-	-	-	No spore	+	*Lactobacillus* sp
NP6	Yellow, Circular, Small-large	Rod	+	+	-	-	-	No spore	+	*Pediococcus* sp
NT3	Yellow, Circular,	Rod	+	-	-	-	-	No spore	-	*Lactobacillus* sp

	Small									
NT5	Creamy, Circular, Large	Rod	+	+	-	-	-	No spore	+	*Pediococcus* sp
NT6	Yellow, Circular, Small	Cocci	+	-	-	-	+	No spore	+	*Streptococcus* sp
NT7	Yellow, Circular, Large	Cocci	+	-	+	-	-	No spore	+	*Streptococcus* sp
NT8	White, Irregular, Large	Rod shape	+	-	-	-	-	No spore	+	*Lactobacillus* sp
NT10	White, Circular, Small	Rod shape	+	-	-	+	-	No spore	+	*Lactobacillus* sp
M05	Creamy, Circular, Small	Oval	+	-	-	-	+	No spore	+	*Streptococcus* sp
MP3	Creamy, Irregular, Small-large	Cocci	+	-	-	-	-	No spore	+	*Streptococcus* sp
MP4	Creamy-yellow, Irregular, Large	Rods	+	-	+	-	+	No spore	+	*Lactobacillus* sp

SCREENING OF ISOLATES FOR PROBIOTIC POTENTIALS

The screening processes adopted were preliminary investigations of the isolates on conditions that mimicked the stress conditions found in the gastrointestinal tract of humans and the ability to tolerate high levels of ethanol for fermentation processes.

Tolerance to a high level of ethanol is a unique trait for microorganisms used for industrial processes especially in the production of alcoholic beverages. Thus, having probiotics with this attribute could be of great importance in fermentation processes especially since probiotics are known to be of great importance in our health. The identified bacterial isolates tolerated ethanol concentrations of 10% and 15% but declined gradually at 20% concentration.

ETHANOL TOLERANCE OF BACTERIAL ISOLATES

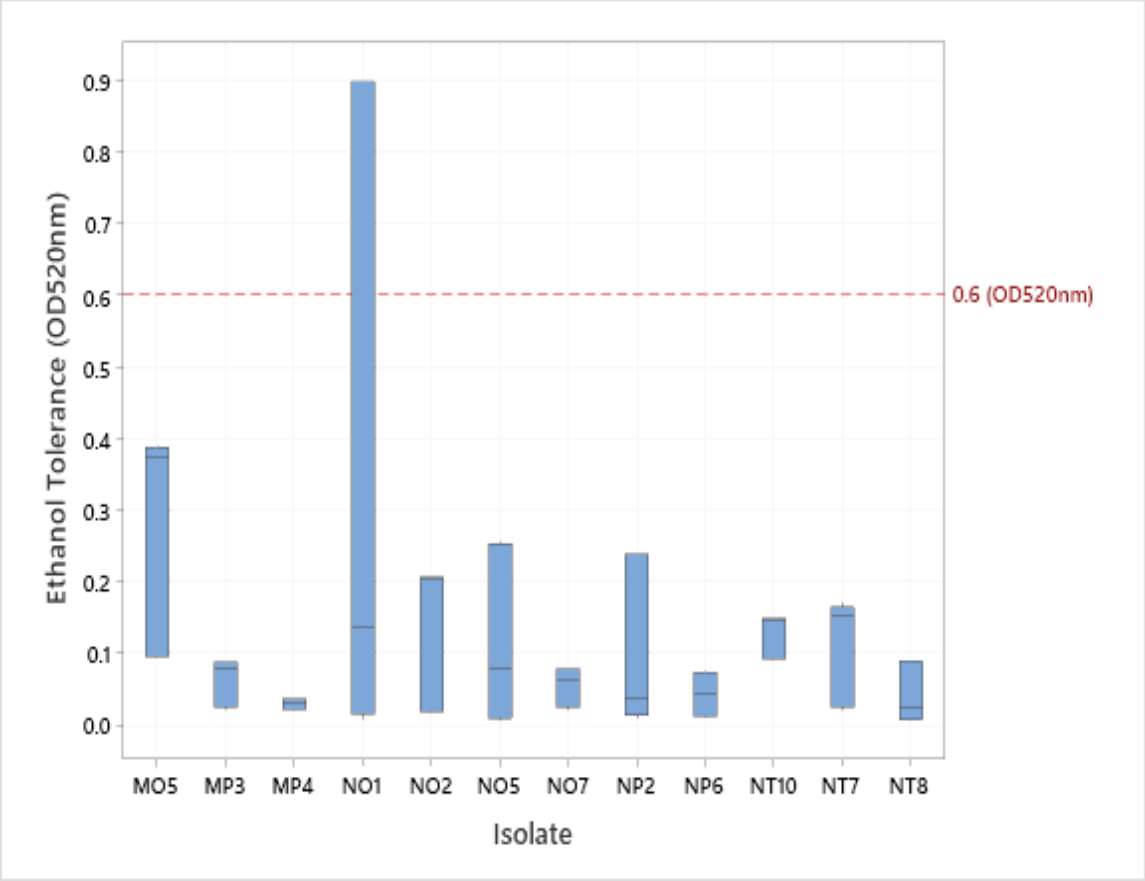

Fig. 1 Ethanol Tolerance of Bacterial Isolates

The result showing the ethanol tolerance of bacterial isolates is presented in fig Results for the bacterial tolerance showed that all the isolates were tolerant (able to grow) at 10% 15% and 20% ethanol concentration but had optimum tolerant range at 15% ethanol concentration (fig. 1). The

ethanol tolerance was carried out to know if these isolates could be used for possible fermentation and preservation of beverages.

BILE SALT TOLERANCE

The bacterial isolates when screened for bile salt tolerance showed that they could withstand varying levels of 1.0, 1.5 and 2.0% concentration. This indicates that these isolates did not only tolerate or withstood the bile salt concentrations but also thrived by doubling their populations after 24 hours of incubation. Although there was a decline in population number in the 2.0% concentration after 48 hours of incubation. According to Singhal *et al.* (2010), many bacteria are inhibited by gastric juice and bile salts, which constitute biological barriers in the stomach and duodenum, respectively. They must also survive transit through the duodenum, where bile salt levels can reach 0.7 percent including reaching the ileum alive, populating it, and passing on their advantages by surviving in these severe stomach circumstances (Byakika *et al.*, 2020). Thus, the bacterial isolates in this study have shown they can tolerate even higher bile salt concentrations. In a previous study, increased viability of the probiotic *L. plantarum* strain GCC_19M1 in 0.3% bile, with a survival rate of 83.70% was reported and despite no significant difference in the growth curve concerning time, there was a marked growth after 3hours of incubation (Nath *et al*., 2020). More so, Khanal and Koirala (2019) reported the ability of some species of *Lactobacillus* to able to survive and multiply in 0.1, 0.3, 0.5 and 1% bile acid. The ability to withstand higher bile concentrations by probiotics is a great feature since bile salts in the body damage the cell membrane's lipids and fatty acids thereby reducing the chances of survival of the organisms (Khagwal *et al.*, 2014). Since bile can disrupt the microbial cellular homeostasis as well as dissociate the lipid bilayer and integrity of the cell membrane resulting in cell death, some lactic acid bacteria in other survive to produce bile salts hydrolase (BSH) which hydrolyzes conjugated bile salts, thus lowering their toxicity (Byakika *et al*., 2020).

Results showing the response of bacterial isolates to bile salt is presented in fig 2. Results showed that all the isolates of bacteria were tolerant at 1.0, 1.5 and 2.0% concentrations of bile salt and the best tolerance was observed in the 1.5% concentration for bacterial.

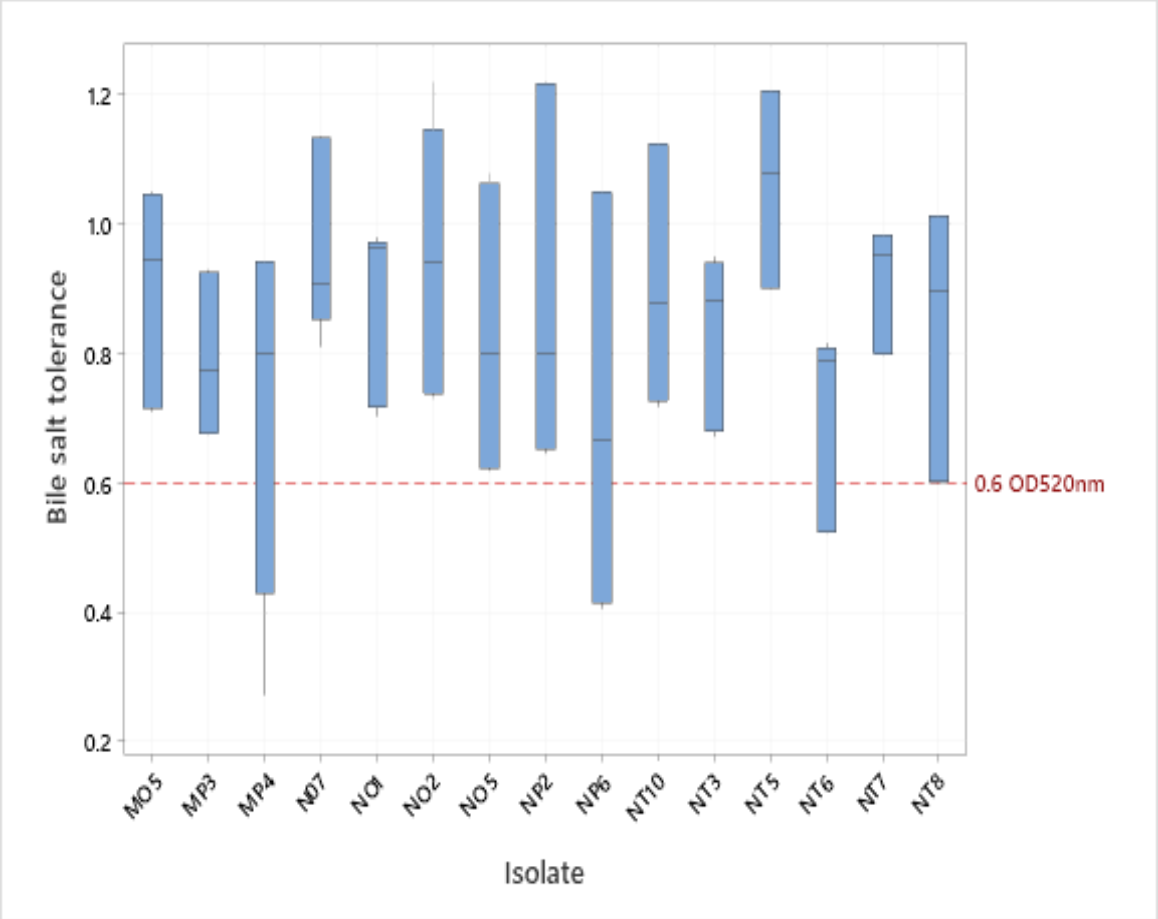

Fig. 2: Tolerance of Bacterial Isolates to Bile Salts

LACTOSE UTILIZATION

Lactose intolerance (LI) is a condition characterized by the inability to digest lactose due to low levels of lactase enzyme activity and over 30 million people have some degree of this condition which is known to decline with age. This condition results in unabsorbed lactose being metabolized by colonic bacteria to produce short-chain fatty acids and gas (Mattar *et al*., 2012). One of the beneficial roles of probiotics in the intestine is to aid in the absorbance or digestion of lactose. The result in this study showed that only 12 bacterial isolates were able to utilize lactose.

The ability of probiotics to utilize lactose has been reported to aid in treating lactose intolerance. In a previous study, L. *bulgaricus* was reported to improve lactose digestion and eliminate the symptoms of intolerance (Oak and Jha, 2018). Also, a study by Zhong (2006) demonstrated that LI symptoms were significantly decreased after *B. animalis* supplementation. Thus, the ability of these bacterial isolates to utilize lactose could mean that they could aid in alleviating the conditions of LI and also improving digestion of lactose in the body.

Results showing the utilization of lactose is presented in Table 2 Out of the fifteen isolates screened for lactose utilization, only twelve isolates: N01, N02, NP2, NP6, NT5, NT6, NT7, NT8, NT10, M05, MP3 and MP4 were lactose utilizers.

Isolate	Response
N01	+
N02	+
N05	-
N07	-
NP2	+
NP6	+
NT3	-
NT5	+
NT6	+
NT7	+
NT8	+
NT10	+
M05	+
MP3	+

MP4	+

Key: - = none lactose utilizer; + = lactose utilizer

Table 3. Lactose Utilization by Bacterial Isolates

BIOGENIC AMINE PRODUCTION

Biogenic amines which are mostly produced by microorganisms such as *S. aureus* and Enterococci are referred to as low-molecular-weight organic bases with strong proteolytic potential for the decarboxylation of histidine, tryptophan, phenylalanine, tyrosine, and lysine amino acids (Moghadam et al., 2021). Hypotension, headache, gastrointestinal problems, flushing, and allergic reactions are all symptoms of high levels of hazardous biogenic amines which have been reported in milk, such as Histamine and Tyramine (Ma *et al.*, 2020). Thus, a good probiotic should not produce biogenic amine. The current study screened both bacterial isolates for the production of biogenic amines. Biogenic amines, which are regarded as one of the undesired biogenic metabolites produced by the starter or probiotic bacteria, must be utilized as a criterion for selecting probiotic strains (Wadu and Rakshit, 2011). It is apparent that identifying and characterizing the microbial strains intended for food fermentation, as well as determining if they are capable of producing BA, to avoid the production of BA and reduce the danger of poisoning. The findings in the current study agreed with the reported data on the biogenic potential of LAB isolated from a wide range of dietary sources (Wadu and Rakshit, 2011). Bunkova *et al.* (2009) identified one strain of *L. delbrueckii* subsp. bulgaricus as possible tyramine former among the thirty-nine LAB examined. The findings of the current study showed that out of the fifteen bacterial isolates screened, only ten (10) which are of the genus *Lactobacillus* and *Pediococcus* sp were negative for the production of biogenic amines.

Results of the screened bacterial isolates for biogenic amine is presented in Table 4 Results showed that out of the fifteen bacterial isolates screened for biogenic amine, only five isolates which include N05, NT3, NT6, M05 and MP3 were positive for the production of biogenic amine while ten isolates which belonged to Lactobacillus *sp* and Pediococcus *sp* did not produce biogenic amine.

Table 4 Production of Biogenic Amine by Bacterial Isolates

Isolates	Response
N01	-
N02	-
N05	+
N07	-

NP2	-
NP6	-
NT3	+
NT5	-
NT6	+
NT7	-
NT8	-
NT10	-
M05	+
MP3	+
MP4	-

Key: + = produced biogenic amine; - = no biogenic amine production.

pH TOLERANCE

The bacterial and yeast isolates in this current study all grew well in the acidic pH of 2. According to Byakika *et al.* (2020), probiotics must survive transit through the stomach, where pH can drop to as low as 3.0, and survive for 2–4 hours. This means that any screened probiotics that cannot withstand the low pH in the stomach might lack the capability of carrying out theirfunctions. The findings in the current study agreed with Nath *et al.* (2020) who reported an analogous growth pattern at pH 3 of *L. plantarum* strain GCC_19M1 during an incubation period of 3 hours.

The result for pH tolerance of bacterial isolates is presented in fig 5 Result showed that the response of the isolates varied significantly despite being able to grow at acidic pH of 2 and that isolates NP6 had the best pH tolerant followed by NP2.

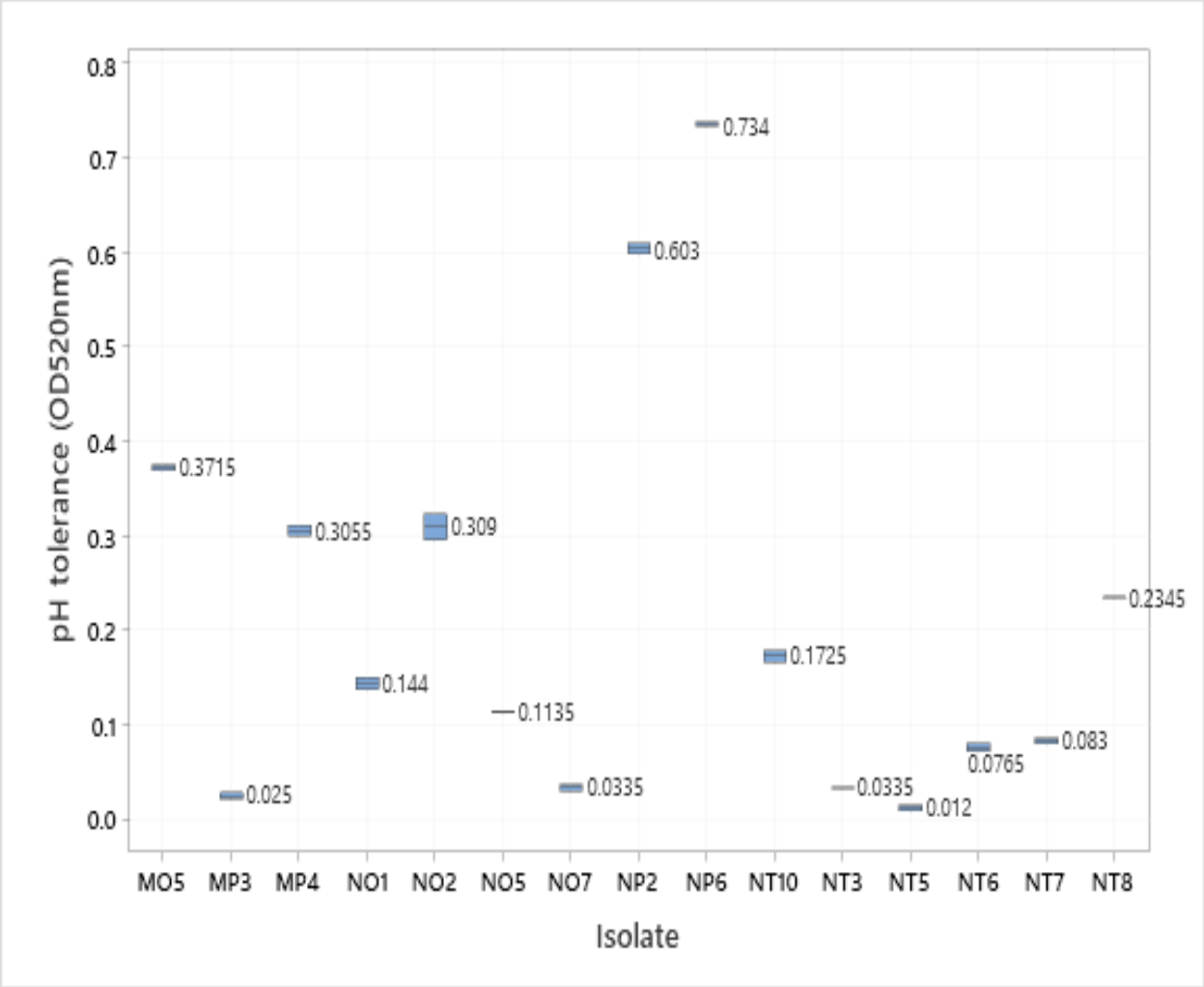

Fig. 3. pH tolerance against isolates

Genomic Characterization of Isolates

Based on the sequencing of the 16S rDNA of the bacterial isolates it was found that bacterial isolates which were characterized phenotypically as *Lactobacillus* sp, and *Pediococcus* sp had close relatedness with *Bacillus cereus* and *Bacillus firmus.* That is isolate NO2 which was phenotypically characterized as *Pediococcus* sp has 83.4% pairwise identity with *Bacillus firmus* strain T1, isolate NP2 which was also identified phenotypically has 86.5% pairwise identity with *Bacillus cereus* strain PKID1 while isolate NT8 previously identified as *Lactobacillus* sp has 80.3% pairwise identity with *Bacillus cereus* strain PV-G21. Molecular characterization is

known as a better tool for characterizing microorganisms especially since it can read the different genetic sequences, unlike the phenotypic method that is mostly characterized by the activities of enzymes that could be controlled by a particular gene. According to Fernández-espinar *et al.* (2011) the use of biochemical methods in the characterization of microorganisms has its advantages especially in expressing the metabolic characteristics of the isolates in question but is not very accurate as some of the expressed biochemical or metabolic responses could be controlled by a particular gene and sometimes there are variations in the physiological response, thus the need for a more precise identification method which targets the genome of the isolates.

Antimicrobial Susceptibility

The antibiotic susceptibility pattern of the screened bacterial isolates showed that they exhibited resistance to Pefloxacin, Gentamycin, Ampiclox, Amoxicillin, Rocephin, Ciprofloxacin; Streptomycin, Sceptrin and Erythromycin. They were highly resistant to Gentamycin, Zinnacef, and Ampiclox. This agreed with Garcia *et al.* (2016) who reported that some probiotics in their study were resistant to erythromycin and gentamycin. Resistance to Ciprofloxacin by the bacterial isolates was also recorded. The natural and intrinsic resistance of Lactobacillus spp. to ciprofloxacin might be attributed to their cell wall structure and membrane impermeability, supplemented in certain cases by potential efflux mechanisms (Ammor *et al.*, 2007). Furthermore, some of the *Lactobacillus* sp and *Streptococcus* sp showed resistance to more than three antibiotics, thus, indicating multi-drug resistance. Sharma et al. (2016) reported that most of the probiotic isolates in their study exhibited multiple resistance against some commonly used antibiotics and that resistance was especially high towards nalidixic acid, nitrofurantoin, kanamycin, teicoplanin, cotrimoxazole, amikacin, streptomycin, norfloxacin, vancomycin, and cefepime. Antibiotic resistance in probiotic bacteria is an increasing issue and antibiotic

resistance genes may be found in probiotic bacteria which can then be passed on to pathogenic bacteria (Temmerman *et al.,* 2003). Antibiotic resistance in pathogens is becoming more of a medical issue as a result, antibiotic susceptibility test should be included in the safety evaluation of desired characteristic of probiotics (Khanal and Koirala, 2019). The findings in the current study do not agree with previous reports in which the probiotics were all susceptible to the antibiotics with the exception of ciprofloxacin (Khanal and Koirala, 2019).

CONCLUSION

The findings in this study showed that tiger nut drink, ogi and palm wine contains probiotics. Although the isolates were phenotypically identified as *Lactobacillus, Pediococcus, Streptococcus* genomic characterization revealed that they were *Bacillus firmus* strain T1, *Bacillus cereus* strain PKID1 and *Bacillus cereus* strain PV-G21

Generally, the screened isolates of bacteria exhibited the required characteristics of probiotics: tolerance to acidic conditions, high salt concentrations, bile salt concentration as well as not showing any sign of pathogenic contamination. More so, the production of biogenic amine by some isolates in these beverages was insignificant since the majority of the isolates were none, biogenic amine producers. The study is in support of other studies which advocates for molecular characterization and fingerprinting of isolates for better identification of probiotics rather than the use of phenotypic characterization alone.

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