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Identification of Lactic Acid Bacteria Isolated from Nigerian Foods: Medical Importance and Comparison of Their Bacteriocins Activities

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ABSTRACT
Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. The genera *Lactobacillus*, *Lactococcus*, *Leuconostoc, Pediococcus*, and *Streptococcus* are important members of this group. The taxonomy of lactic acid bacteria has been based on the Gram reaction and the production of lactic acid from various fermentable carbohydrates.The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, and configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance. Based on sugar fermentation patterns, LAB are classified into homofermenters and heterofermenters. Lactic acid bacteria have been used in the treatment of infections and in the production of foods, especially fermented foods (such as yoghurt, Cheese, etc.) because they can produce several compounds that contribute to taste, smell, color, and texture of the foods. In addition, they can produce antimicrobial substances including bacteriocins. LAB have the ability to inhibit pathogenic and food spoilage bacteria. The bacteriocins synthesized by different lactic acid bacteria inhibited the growth of the test organisms used and this was observed through the production of zones of inhibition on the plates.
Keywords: Generally regarded as save (GRAS), bacteriocin, MRS agar, nisin, lactic acid bacteria (LAB), heterofermenter, homofermenters, B*ifidobacterium,* acidophiline, bulgarican

Introduction

Lactic acid bacteria are a group of related bacteria that produce lactic acid as a result of carbohydrate fermentation. These microbes are broadly used by us in the production of fermented food products, such as yogurt (*Streptococcus spp.* and *Lactobacillus spp.*), cheeses (*Lactococcus spp.*), sauerkraut (*Leuconostoc spp.*) and sausage.

Recent taxonomic revisions suggest that lactic acid bacteria comprise the following genera: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella* [1]. Orla-jenson classified LAB according to morphology; rods and cocci, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentration acid or alkaline tolerance, configuration of the lactic acid produced. Based on sugar fermentation patterns, two broad categories are known homofermenter and heterofermenter.

Homofermenters are LAB that convert hexoses to produce almost quantitatively lactic acid, E.g. *Enterococcus faecalis, Lactobacillus acidophilus, Streptococcus bovis, Lactococci spp* and Vagococci spp, etc. Heterofermenters are LAB that convert sugars to lactic acid, ethanol and CO_2 they include *Lactobacillus brevis, Lactobacillus fermentum, Leuconostoc dextranicum, Weissella spp, etc.*

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{lactic acid}}{2\,CH_3CHOHCOOH}$$

Homofermentation of 1 mole of glucose yields two moles of lactic acid

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{lactic acid}}{CH_3CHOHCOOH} + \underset{\text{Ethanol}}{C_2H_5OH} + \underset{\text{carbon dioxide}}{CO_2}$$

Table 1: Heterofermentation of 1 mole of glucose yields 1 mole each of lactic acid, ethanol and carbon dioxide

Most species have multiple requirements for amino acids and vitamins. Because of this, lactic acid bacteria are generally abundant only in communities where these requirements can be provided. They are often associated with animal oral cavities and intestines (e.g. *Enterococcus faecalis*), plant leaves (*Lactobacillus, Leuconostoc*) as well as decaying plant or animal matter such as rotting vegetables, fecal matter, compost, etc.

Lactic acid bacteria are used in the food industry for several reasons. Their growth lowers both the carbohydrate content of the foods that they ferment, and the pH due to lactic acid production. It is this acidification process which is one of the most desirable side-effects of their growth. The pH may drop to 4.0, low enough to inhibit the growth of most other microorganisms including the most common human pathogens, thus allowing these foods prolonged shelf life. The acidity also changes the texture of the foods due to precipitation of some proteins, and the biochemical conversions involved in growth enhance the flavour. The fermentation is self-limiting due to the sensitivity of lactic acid bacteria to such acidic pH. Most are free-living or live in beneficial associations with animals, although some are opportunistic pathogens. They are normal flora of man in the oral cavity, intestinal tract and vagina, where they play a beneficial role.

In 2008, Axelsson highlighted a few pathogenic LAB for animals, most notably some members of the genus *Streptococcus*. In humans, *Streptococcus pyogenes* is a major cause of disease (strep throat, pneumonia, and other pyogenic infections, scarlet fever and other toxemias), *Streptococcus pneumoniae* causes lobar pneumonia, otitis media and meningitis; some *Viridans* and non-haemolytic oral *Streptococci* play a role in dental caries and may be an insidious cause of endocarditis [2].

Some strains of *Lactobacillus* spp. and other lactic acid bacteria may possess potential therapeutic properties including anti-inflammatory and anti-cancer activities, as well as other features of interest. A study by researchers from the Beth Israel Deaconess Medical Center and UCLA in 2009 demonstrated the protective effects of some strains of these bacteria for anti-tumor and anti-cancer effects. Dietary administration alleviated the risks of certain types of cancers and suppressed colonic tumor incidence, volume and multiplicity induced by various carcinogens in mice. For a few strains oral administration effectively reduced DNA adduct formation, ameliorated DNA damage and prevented putative preneoplastic lesions such as aberrant crypt foci induced by chemical carcinogens in the gastrointestinal tract. Reports also indicated that some cultures administered to animals inhibited liver, colon, bladder, and mammary tumors, highlighting potential systemic effects of probiotics with anti-neoplastic activities [3].

Lactobacilli can also be used to restore particular physiological balance such as in the vaginal eco-system. Their role is to physically protect the vaginal epithelium by building a thick layer separating the epithelium from pathogens [4] also to physiologically keep the balance of the vaginal ecosystem in maintaining the pH at ~ 4.5 [5] and generating hydrogen peroxide against pathogens. *Lactobacilli* are highly tolerant to low pH and can easily maintain low pH and protect the vaginal eco-system from Gram-negative and Gram-positive bacteria [6]. Some *Lactobacillus* species have been associated with dental caries. The *Lactobacillus* count in saliva has been used as a "caries test" for many years. This is one of the arguments used in support of the use of fluoride in toothpaste and lozenges [7]. *Lactobacilli* characteristically cause existing carious lesions to progress, especially those in coronal caries.

The bacteriocinogenic strain *Lactobacillus plantarum* isolated from the traditional pearl millet-based African fermented food "ben saalga" was tested for inhibition of food poisoning and pathogenic bacteria in MRS broth and in malted millet flour slurry. In MRS broth, strain 2.9 completely eliminated *Bacillus cereus, Escherichia coli* O157:H7 and *Salmonella enterica* cells within 48 h incubation at 22–30 °C. A much lower inhibition was observed at 15 °C. The inhibitory effect of strain 2.9 on the above-mentioned target bacteria was corroborated in the malted millet flour slurry, reducing viable cell counts below detection levels after 8 h storage for *B. cereus* or after 24 h for *S. enterica* and 48 h for *E. coli.*

MEDICAL IMPORTANCE TO MAN

Essential Companions for man

L. acidophilus and *Bifidobacterium* exist in the intestine and, which exists from the lower part of the small intestine to the large intestine. These microorganisms have the specific property of transforming sugars almost exclusively into lactic acid and acetic acid that decrease the pH (increasing the acidity) of the intestines and produce substances that suppress harmful bacteria. They are abundant in nature and are essential for human and animal survival [8]

Prophylaxis and therapeutic treatment

Hatakka *et al*, (2001) reported that the normal intestinal flora is constituted from groups of microorganisms among which lactic bacteria perform essential functions:

i. Transforms glucose into lactic acid, creating a favorable environment for the desirable microbial balance.
ii. Limits the action of putrefactive microbes.
iii. Inhibits the development of pathogenic bacteria.
iv. Hydrolyses lactose and eliminates the intolerance of the organism toward this sugar.
v. Contributes to intestinal peristalsis and accelerates the evacuation of excrements.
vi. Coats the intestinal mucosa and protects it against the invasion of harmful microorganisms

vii. Activates macrophages (immune cells) that suppress harmful bacteria and thus protect the intestines

A fragile balance

Many factors can modify the desirable harmony of the intestinal flora. Disease, stress, the abundance of food proteins, the consumption of contaminated food and the taking of antibiotics can favour the implantation and development of putrefactive and infectious microorganisms to the detriment of desirable bacteria. The prolonged use of antibiotics not only has the effect of destroying lactic bacteria from the digestive system, leaving it without defense, but it provokes the development and predominance of harmful microorganisms such as coliforms and pathogenic *Staphylococci*.[23]

Gastro-intestinal infections

i. Gastro-intestinal infections originating from bacteria are generally due to a restricted number of species of bacteria belonging to *Escherichia, Shigella, Salmonella, Klabsiella, Proteus* and *Staphylococci*. [14] These infections are more frequently found in children and can be the result of accidental contamination from impure water or food or from the transmission of infectious microbes from other individuals or from infected places.

ii. An important source of infectious bacteria is made by the creation of resistant pathogenic colonies due to the prolonged administration of weak doses of antibiotics. This particular resistance present in *Escherichia coli* can be genetically transmitted to other kinds of *Enterobacteria* belonging to the *Shigella, Salmonella, Klabsiella* and *Proteus* [9]. These genetic modifications take on considerable importance because they multiply the possibilities for infection and complicate the administration of effective medication.

Vaginitis

Lactic bacteria assure the protection of the vagina [10], especially by lactobacilli. They were originally designated by the name *Doderlein bacillus*, but we now know that this bacterium belongs to several species, that include *Lactobacillus acidophilus, L. bifidus, L. rhamnosus, L. fermenti, L. plantarum,* etc. For many reasons, the administration of antibiotics, hormonal troubles, inadequate enemas, contact with infected individuals, and lack of resistance due to *Candida albicans*, to *Trichonomas vaginitis* or to infectious bacteria may develop. One of the successful treatments utilizes appropriate lactobacilli. Introduced by an enema, it restores the normal lactic flora, lowers the pH of vaginal secretions to a desirable level (pH4.5) and inhibits the existing infection.

Intestinal putrefaction

After more than 85 years of hesitation and of denial by orthodox medicine, it is now recognized that Metchnikoff was right when he stated that intestinal putrefaction was the source of many diseases and constituted an attack more or less rapid on the vitality and longevity of the human being.

i. The intestinal putrefaction, resulting from the activity of putrefactive bacteria, expresses itself by the development of a great variety of toxic substances that include several acids, ammonia, hydrogen sulfide, amides, methylated amines, methane, indole, phenol, mercaptans, etc.

ii. The organism is not always able to defend itself against the activities of harmful microorganisms. Several organs may be attacked. The secretion of digestive enzymes is hindered, the kidneys become impervious, the endocrine system weakens, and the suprarenal are attacked. This results in serious and diversified organic disorders that may lead to debility, atherosclerosis and early senility.

iii. Recently, it has been shown that a tangible effect from intestinal putrefaction is recognizing that hepatic encephalopathy was caused by the breakdown of nitrogenous substances in the lower intestine and the absorption of toxic products, especially ammonia and amines. The orthodox treatment of this sickness is by enemas and laxatives, administration of antibiotics and even surgical removal of the colon with the goal of interrupting the activity of putrefactive bacteria [11]. A group of scientists has attempted to modify the intestinal flora of patients attacked by hepatic encephalopathy by administrating to them *Lactobacillus acidophilus*. In suppressing the harmful flora, there was a reduction in phenomena of deamination and the level of ammonia in the blood of the patients. Many other scientists have confirmed these discoveries subsequently

Reduces gas and bloating

Lactose intolerance is caused in part by a shortage of lactase, an enzyme produced by the cells that line the small intestine. Lactase breaks lactose down into the simple forms of sugar, glucose and galactose, so they can be absorbed and used by the body. Lactic acid bacteria in the intestines break lactose down into short-chain fatty acids and other substances that can be absorbed by the colon. Lactose left undigested in the intestines can result in diarrhea, because of the excessive amounts of water that are drawn into the intestines by lactose. Hydrogen is produced, causing gas and bloating.

Inhibits the growth of pathogenic bacteria

Lactic acid bacteria inhibit the growth of pathogenic bacteria [10] and their production of toxins. *L. acidophilus* has a superior capability of producing lactic acid as well as anti-bacterial substances, thus suppressing harmful bacteria. *Bifidobacterium* particularly protects the body from harmful bacteria by adhering to the intestinal mucosa (cells lining the intestines), by producing acetic acid and by activating macrophages (immune cells) that also produce substances that suppress harmful bacteria.

Decreases the production of toxic and cancer-causing compounds in the intestinal tract

Lactic acid bacteria suppress the production of harmful substances such as ammonia, indole and hydrogen sulfide that are hazardous to the human body. [11] *Bifidobacterium* helps to decrease the amount of toxins going to the liver. Gut bacteria "recycle" toxins such as ammonia by using it as an important source of nitrogen for their own protein synthesis during their growth phase. *Bifidobacterium* and *L. acidophilus* bacteria decompose nitrosamines (cancer causing compounds) and can also suppress the production of nitrosamines in the intestines.

Impact on cholesterol

Bifidobacterium and *L. acidophilus* may play an important role in cholesterol metabolism of their host. Intestinal bacteria convert cholesterol into a less absorbable form (coprostanol) thus hampering its absorption from the intestinal tract. Several human and animal studies [8] have suggested a cholesterol lowering effect from lactic acid bacteria.

Produce vitamins and other nutritional factors

Bifidobacterium have shown an ability to produce vitamin B_1, whereas acidophilus bacteria have been shown to suppress the growth of bacteria that decompose vitamin B_1. *Bifidobacteria* are also capable of producing B_6, folacin, B_{12} and several amino acids. [15]

The production of specific antibiotics

Lactobacillus acidophilus produces acidophiline, *L bulgaricus* produces bulgarican, [6] other lactobacilli produce lactocidine, lactobacilline, hydrogen peroxide, bacterial peptides and lactic streptococci produce nisin and streptococcins. Modern methods of investigation have demonstrated the intense colonization of intestinal mucosa by lactic bacteria, in particular by *Streptococcus faecium*. This colonization protects the intestinal wall by creating a barrier against infectious microbes.

LACTIC ACID BACTERIOCIN

Currently the major interest in using lactic bacteria is that the undesirable microorganisms in foods may be inhibited by one of the bacteriocin-producing lactic acid bacteria. Bacteriocins are microbial compounds of a proteic nature that have a bactericidal or bacteriostatic effect on other closely related species [12], [13]. Many types of bacteriocins have been characterized and they have considerable potential for application in foods, aiming at the quality and safety of these foods [14]. Some bacteriocins produced by lactic acid bacteria, such as nisin, inhibit not only closely related species but are also effective against food-borne pathogens and many other Gram-positive spoilage microorganisms [15]. For this reason, bacteriocins have attracted considerable interest for use as natural food preservatives in recent years, which have led to the discovery of an ever increasing potential source of these protein inhibitors.

Since bacteriocins are isolated from foods such as meat and dairy products, which normally contain lactic acid bacteria, they have unknowingly been consumed for centuries. A study of 40 Wide-type strains of *Lactococcus lactic* showed that 35 produced nisin [16]. Nisin is the only bacteriocin with GRAS (Generally Regarded as Safe) status for use in specific foods and this was awarded as a result of a history of 25 years of safe use in many European countries and was further supported by the accumulated data indicating its nontoxic, non-allergenic nature. Other bacteriocins without GRAS status will require pre-market approval. Therefore, bacteriocinogenic starters, particularly if used in natural fermentations, will most likely afford the best opportunities for the application of bacteriocins in near future.

The target of bacteriocins is the cytoplasmic membrane and because of the protective barrier provided by the LPS of the outer membrane of Gram-negative bacteria, they are generally only active against Gram-positive cells [17, 18]. In the context of fermentation, important targets include spoilers such as species of *Clostridium* and foodborne pathogens including *Listeria monocytogenes*, *Staphylococcus* spp., *Clostridium, Enterococcus,* and *Bacillus* spp. The permeability of Gram-negative bacteria can be increased by sublethal injury including that which can occur when using ultrahigh hydrostatic pressure (UHP) and pulsed electric field (PEF) as nonthermal methods of preservation [19]. In addition, disruption of the integrity of the outer membrane through the use of food grade chelating agents such as ethylenediamine tetraacetic acid (EDTA) and citrate which bind magnesium ions in the LPS layer can increase the effectiveness of bacteriocins against Gram-negative bacteria [20]. Many bacteriocins are most active at low pH [21] and there is evidence that bacteriocinogenic strains can be readily isolated from fresh and fermented foods [22]. Strains may naturally produce more than one bacteriocin and heterologous expression of bacteriocins has been demonstrated in constructed strains [23]. Protein engineering has led to the development of nisin derivatives with altered antimicrobial activities or greater solubility at pH 6 than the wild-type nisin [24]. An advantage of bacteriocins over classical antibiotics is that digestive enzymes destroy them.

Bacteriocin producing strains can be used as part of or adjuncts to starter cultures for fermented foods in order to improve safety and quality. Bacteriocins of lactic acid bacteria, according to the classification procedure proposed by Klaenhammer and modified by Nes *et al*, (1987), are divided into four classes as shown in Table 2. The majority of those produced by bacteria associated with food belong to classes I and II. Some of bacteriocins isolated from lactobacilli are listed in Table 3. Most of them belong to the class II bacteriocins [25].

Table 2: Classes of bacteriocins produced by lactic acid bacteria

Class	Subclass	Description
I		Lantibiotics-small, heat stable, containing unusual amino acid
II		Small (30-100 amino acids), heat stable, non-lantibiotic
	IIa	Pediocin-like bacteriocins, with anti-listerial effects
	IIb	Two peptide bacteriocins
III		Large (>30 kilodaltons; kDa) heat-labile proteins
IV		Complex bacteriocins with glycol-and/or lipid moieties

Table 3: Examples of bacteriocins of *Lactobacillus* species

Producer Strain	Bacteriocin	Spectrum
L. sake	Lactocin S	*Lactobacillus* spp. *Leuconostoc* spp. *Pediococcus* spp.
L. sake	Sakacin P	*Lactobacillus* spp. *Carnobacterium* spp.
L. sake	Sakadin A	*Lactobacillus* spp. *Carnobacterium piscicola* *Enterococcus* spp. *Listeria monocytogenes*
L. bavaricus	Bavaricin A	*Lactobacillus* spp. *Lactococcus* spp. *Pediococcus* spp. *Enterococcus* spp. *Listeria monocytogenes*
L. acidophilus	Lactacin F	*Lactobacillus* spp. *Enterococcus faecalis*
L. curvatus	Curvacin A	*Lactobacillus* spp. *Carnobacterium* spp. *Listeria monocytogenes*
L. helveticus	Helveticin J	*L. helveticus* *L. bulgaricus* *L. lactis*

Table 4: Antagonistic activities caused by lactic acid bacteria

Metabolic product	Mode of antagonistic action
Carbon dioxide	Inhibits decarboxylation? Reduces membrane permeability?
Diacetyl	Interacts with arginine-binding proteins.
Hydrogen peroxide / Lactoperoxidase	Oxidizes basic proteins.
Lactic acid	Undissociated lactic acid penetrates the membranes, lowering the intracellular pH. It also interferes with metabolic processes such as oxidative phosphorylation.
Bacteriocins	Affect membranes, DNA-synthesis and protein synthesis.

COMPARISON OF THEIR BACTERIOCINS ACTIVITIES

Materials and method

Samples collection/collection of samples:

The sample of fura used for this study was obtained from the Fulani village behind crown estate, Okada, Edo State, Nigeria while the Wara and Nunu were collected directly from the Fulani's living in neighboring village around Ore town in Ondo State Nigeria. To maintain its originality, sterile sample bottles were used to collect the samples and immediately kept in the laboratory refrigerator at 5^oC for further analysis.

Serial dilution of the samples

Stock solutions of 10% of fermented fura, nunu and wara was prepared by dissolving 1Gram each of the samples in 10ml of sterile peptone water in a test tube, this was further serially diluted to obtain concentrations of 10^{-4}, 0.2 ml of 10^{-4} dilution from each sample was transferred into 20 ml of sterile DeMann, Rogosa and Sharpe agar (MRS) medium, it was thoroughly mixed before pour plating. The plates of wara, fura and nunu labeled as 'W, F and N' respectively were allowed to set and incubated anaerobically at 32^oC for 24 hrs and the different colonies of the bacteria observed.

Isolation and identification of the bacteria

Distinct and well isolated colonies were sub-cultured and examined for various sizes, shapes, colours and texture, a series of tests such as catalase, oxidase, indole, Nitrate reduction, and sugar fermentation were carried out to identify the bacteria.

Catalase test

From the pure culture, a catalase test was conducted. This biochemical test is used to detect the presence of the enzyme catalase, which is present in aerotolerant aerobic bacteria. An end product of aerobic respiration is the toxic compound hydrogen peroxide. The enzyme catalase reduces hydrogen peroxide to water and oxygen, and may be represented by the following balanced equation:

$$H_2O_2 \xrightarrow[\text{catalase}]{} 2H_2O + O_2$$

Gram staining

A Gram stain was conducted on the pure culture. This stain was used to determine the makeup of the cell membrane of bacteria.

Spore staining

Using a pure colony of the *Lactobacillus* control, a spore stain was conducted according to (Microbiological Methods, 2007). The spore stain is used to determine whether the bacteria produce spores or not.

Growth at 4% NaCl

An overnight broth culture of the isolate was prepared with 4% NaCl added to it and the broth solution was observed to be turbid after a period of 24 hours.

Motility test

This is used to check for the ability of bacteria to migrate away from a line of inoculation.

Test Bacteria

The test organisms used in this study were kindly provided by University Teaching Hospital, Ibadan and designated as follows: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella* spp, *Pseudomonas aeruginosa* and *Proteus mirabilis.* The bacteria were grown on differential media and selective media. Distinct and well isolated colonies were sub-cultured. Microscopy was performed for various sizes, shapes, colours and texture, a series of biochemical tests such as catalase, oxidase, indole, MRVP, citrate and urease to identify the organisms

Detection of antimicrobial activity.

Eight test-tubes of 5ml each of MRS broth were prepared and inoculated with eight isolates obtained from the three samples and labeled as W1, W2, W3, F1, F2, N1, N2 and N3. After 24hrs, they were then centrifuged at 3000 rpm for 15 minutes the more dense organelles settle at the bottom of the test tube while the supernatant that contains the bacteriocin forms the upper layer. The antimicrobial assay was performed using the agar well diffusion described by Schillinger and Lucke (1989).The bacteria were seeded into the molten agar poured in plates and left to set, 0.2ml of bacteriocin obtained was introduced into the wells bored with 5 mm cork borer. The plates were incubated right side up at 32^0C for 24hrs after which the plates were examined for zones of inhibition. Three replicates were made for each bacteria and the average activity was recorded. The antimicrobial activity of the bacteriocins was compared with that of Gentamicin

RESULTS

Description of bacterial growth on plates

There were various distinct colonial morphologies ranging from cream to white on the plates, the colonies were small, smooth, whereas the cream microbes formed smaller grainy-looking colonies as shown below

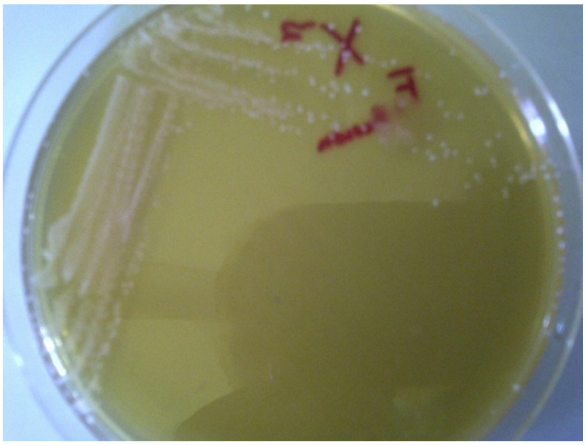
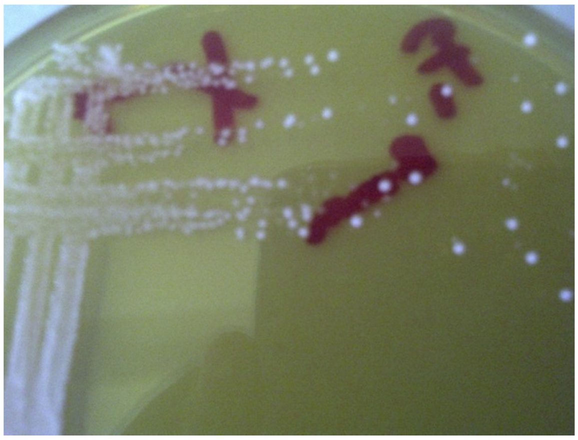

Plate1: colonies appearance on MRS agar

A total of 8 isolates were obtained based on their colonial/morphological and biochemical activities, they are shown in Tables 5 & 6 below.

Table 5: morphological characteristics

Isolates	**Color**	**Shapes**	**Morphology**
W1	white	rods	Clustered chains
W2	Dirty-white	cocci	Clustered straight
W3	Dirty-white	cocci	Clustered
F1	Cream	cocci	Thick, spherical, chain
F2	white	rods	Thick, short rods, chain
N1	white	rods	Chains in groups
N2	cream	cocci	Long cocci chains
N3	white	rods	Thick but short bacilli

Table 6: Biochemical activities

Isolate	Gram stain	Catalase Test	Coagulase Test	Indole Test	Oxidase Test	Spore Test	Motility Test	Growth at 4% NaCl
W1	+	-	-	-	-	-	-	+
W2	+	-	-	-	-	-	-	+
W3	+	-	-	-	-	-	-	+
F1	+	-	-	-	-	-	-	+
F2	+	-	-	-	-	-	-	+
N1	+	-	-	-	-	-	-	+
N2	+	-	-	-	-	-	-	+
N3	+	-	-	-	-	-	-	+

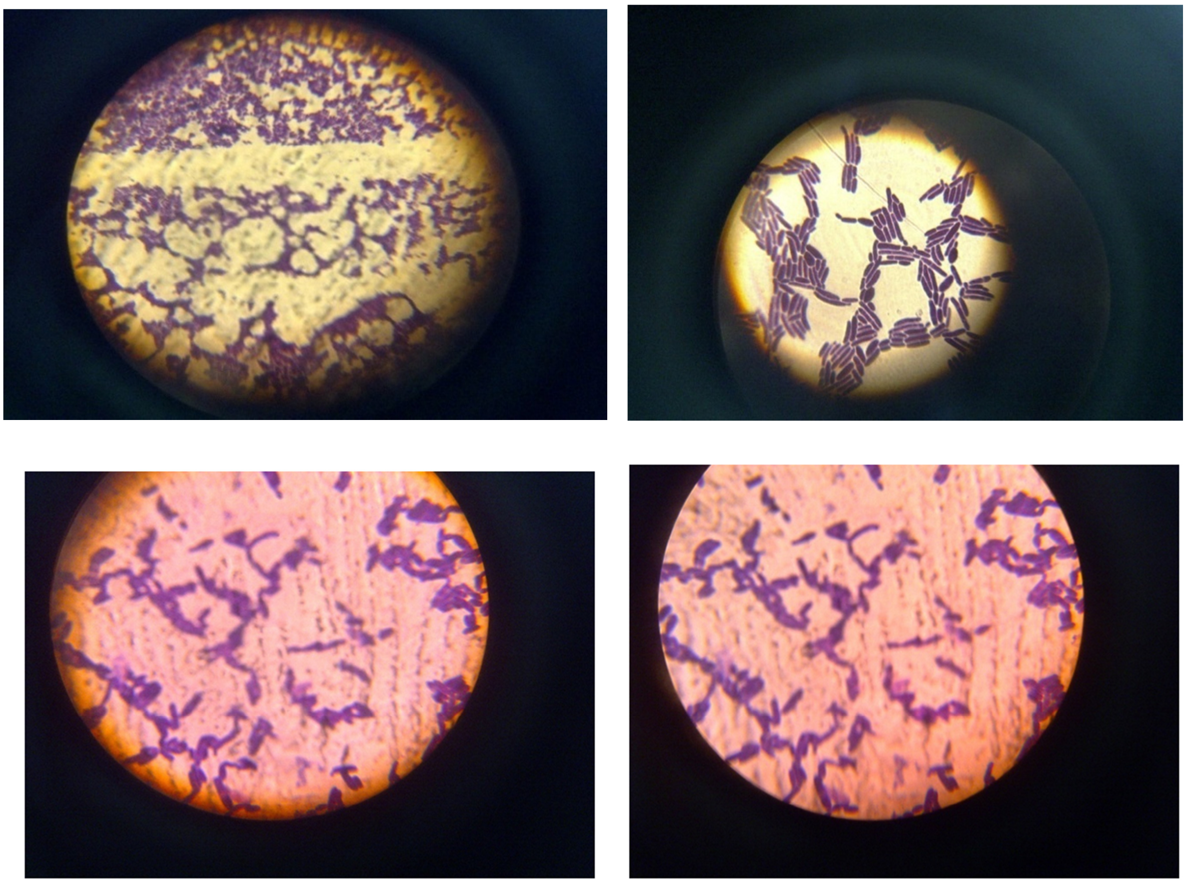

Plate 2: Microscopical Morphology of some of the isolates

Differential characteristics of lactic acid bacteria

Table 8: result of sugar fermentation

	ISOLATES							
sugars	W1	W2	W3	F1	F2	NI	N2	N3
Gelatin	-	-	-	-	-	-	-	
glycerol	-	-	-	-	-	-	-	-
ribose	-	+	+	+	+	-	+	-
D-xylose	-	+	+	-	+	-	+	-
D-glucose	+	+	+	+	+	+	-	+
D-fructose	+	+	+	+	+	+	+	+
D-mannose	+	+	+	-	+	-	+	+
N-A.glucosamine	+	+	-	-	+	-	-	-
Amygdalin	-	-	+	+	+	-	-	-
Salicine	+	+	+	-	+	-	+	-
Manitol	-	-	-	+	+	-	-	-
Maltose	+	-	+	+	+	-	-	+
Lactose	-	-	-	+	+	+	-	+
Galactose	+	+	+	+	+	-	-	-
Trehalose	+	+	-	+	+	-	+	-
Saccharose	+	-	+	-	+	-	-	+

NAMES OF SUSPECTED LAB

KEY
W1 *Lactobacillus spp*
W2 *Leuconostoc spp*
W3 *Lactococcus spp*
F1 *Leuconostoc spp*
F2 *Lactobacillius spp*
N1 *Lactobacillus spp*
N2 *Leuconostoc spp*
N3 *Lactobacillus spp*
The table below shows the results of susceptibility tests of various bacteriocins extracted from LAB

Table 9: Sensitivity to bacteriocin

	ZONE OF INHIBITION(mm)							
Test organisms	*Lactobacillus spp* **(W1)**	*Leuconostoc spp* **(W2)**	*Lactococcus spp* **(W3)**	*Leuconostoc spp* **(F1)**	*Lactobacillius spp* **(F2)**	*Lactobacilus spp* **(N1)**	*Leuconostoc spp* **(N2)**	*Lactobacillus spp* **(N3)**
Bacillus subtilis,	11	-	-	-	10	-	-	14
Staphylococcus aureus,	14	12	9	-	9	12	13	12
Escherichia coli,	-	10	-	-	12	10	-	12
Klebsiella spp,	12	12	-	9	-	9	12	10
Pseudomonas aeruginosa	-	-	-	-	10	-	-	-
Proteus mirabilis	10	11	-	-	13	12	13	11

N.B: Values represent mean of duplicates.
-- means no inhibition

DISCUSSION
From the table above, there were zones of inhibition in the various bacteriocins and this indicates the efficacy of these toxins on the bacteria used. *B. subtilis* was only sensitive to toxin of *Lactobacillus spp* isolated from wara, fura and nunu and resistant to the other bacteriocins. In all the bacteria used, *S. aureus* was seen to be most susceptible to the bacteriocins, there was zones of inhibition ranging from 9-14mm except in the bacteriocin of *Leuconostoc spp* where it was resistant. *E.coli* on the other hand was resistant to bacteriocins of *Lactobacillus* and *Lactococcus* of wara, *Leuconostoc* of wara and nunu respectively but susceptible to the other bacteriocins with varied zones of inhibition. The bacteriocins of *Lactococcus* isolated from wara and *Lactobacillus* of fura do not have any antimicrobial effect on *Klebsiella spp*, others bacteriocins were potent. *Pseudomonas aeruginosa* was resistant in all the bacteriocins but susceptible to bacteriocin of *Lactobacillus spp* from fura with zone of inhibition of 10mm. bacteriocins of *Lactococcus spp* from wara and *Leuconostoc spp* from fura does not have any activity on *Proteus mirabilis* but this bacterium was resistant to bacteriocins of *Lactobacillus spp* isolated from wara, fura and nunu, including *Leuconostoc* of wara and nunu. From these result it was observed that bacteriocins of *Lactococcus spp* from wara and *Leuconostoc spp* from fura were least active against the test organisms, while bacteriocins of *Lactobacilli* from fura and nunu were most potent on the organisms.

CONCLUSION
Since the susceptibility test of lactic acid bacteriocins showed good antimicrobial properties, it can therefore be recommended medically for treating patients living with these infections and those receiving radiation treatment, individuals who have recurrent thrush, vaginal yeast infections, or urinary tract infections, persons suffering from irritable bowel syndrome or other bowel problems, for travelers abroad to protect them against food poisoning and during any period where antibiotics may be taken [29]. LAB have displayed numerous antimicrobial activities in fermented foods. This is mainly due to the production of organic acids, but also of other compounds, such as ethanol, H_2O_2, diacetyl, reuterin and bacteriocins. Several bacteriocins with industrial

potential have been purified and characterized. Application of bacteriocin-producing starter cultures in fermented foods has been studied during *in vitro* laboratory fermentations as well as on pilot-scale level. The promising results of these studies underlined the important role that bacteriocinogenic lactic acid bacteria may play medically and in food industry.

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