Original Research Article

Preliminary study on antimicrobial activity of friendly bacteria isolated from dairy products

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Lactic acid bacteria (LAB) are therefore excellent ambassadors for an often maligned microbial world, they are not only of major economic significance, but also of value in maintaining and promoting human health. However fifteen Lactic acid bacteria were isolated for the purpose of this work from milk and milk product, they were identified as Lactobacillus plantarum, Lactobacillus fermentum, Lactococcus lactis subsp. cremoris, Lactobacillus acidophilus, Lactobacillus brevis and Streptococcus thermophilus. Lactobacillus plantarum produced the highest quantity of Lactic acid at 72 hrs of incubation (2.89g/l) while Lactobacillus fermentum followed closely (1.11g/l), Lactococcus lactis subsp. cremoris had the highest quantity of diacetyl (1.72g/l) while Streptococcus thermophilus and Lactobacillus fermentum had the lowest quantity of 0.52g/l and 0.47g/l. At 72hrs of incubation Lactic acid produced the highest quantity of hydrogen peroxide (1.91g/l) while Streptococcus thermophilus produced the lowest, the quantity of hydrogen peroxide increases up to 48hrs before declination sets in. Some alleged health benefits of consuming LAB, with Significant researches back up the claims that LAB enhance specific and nonspecific immune response, inhibit pathogen growth and translocation, stimulate gastrointestinal immunity, reduce chance of infection from common pathogens (Salmonella, Shigella), reduce risk of certain cancers (colon, bladder) Detoxify carcinogens Lower serum cholesterol concentrations improve urogenital health to name just but a few. However this paper tend to summarise the positive impact of LAB in health maintenance and disease prevention due to the antimicrobials produced by the organisms and extensive study that have been carried out by various researchers on these organism generally regarded as safe hence the name "The Good Guys"

Key words: GRAS LAB, Probiotic, Lactobacillus plantarum.

INTRODUCTION

Microbial cultures have been used for many years in food and alcoholic fermentations, and in the past century have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. Without understanding the scientific basis, people many years ago used lactic acid bacteria to produce cultured foods with improved preservation properties health attributes and commercial value of industrially and traditionally fermented foods and with characteristic flavours and textures different from the original food. (Jay et al., 2005)

LAB refers to a large group of beneficial bacteria that have similar properties and all produce lactic acid as an end product of the fermentation process. They are widely distributed in intestinal tracts of various animals where they live as normal flora. The largest genus in this order is Lactobacillus which contains almost 80 species and are used in differentiation products such as pickle, sauerkraut, beer, wine, juices, cheese, yogurt, and sausage (Prescott et al., 2002), (Azizpur, 2009). LAB is facultative anaerobes, non-sporulating, and acid tolerant. They are strictly fermentative and have complex, sometimes very fastidious nutritional requirements for carbohydrates, amino acids,
produce large amounts of lactic acid and small amounts of other compounds are the products of their carbohydrates metabolism. (Jay et al., 2005)

LAB produce various compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocin or bacterial proteins during lactic fermentations (Zhennai, 2000; Oyetayo et al., 2003). The bacteriocins from the generally recognized as safe (GRAS) lactic acid bacteria (LAB) have risen a great deal of attention as a novel approach to control pathogens in food-stuffs. Innovative approaches have been tried as alternative to antibiotics in treating gastrointestinal diseases and these include using live biotherapeutic agent such as bacterial isolates (Oyetayo et al., 2003) Lactic acid bacteria exert strong antagonistic activity against many microorganisms including food spoilage organisms and pathogens. In addition, some strains may contribute to the preservation of fermented foods by producing bacteriocins. Some bacteriocins are also active against Gram-positive food-borne pathogens such as Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis and spores of Clostridium perfringens. For this reason, they have received much attention for use as natural or so-called ‘biopreservatives’ in food in recent years (Savadogo et al., 2006). Research on bacteriocins from lactic acid bacteria has expanded during the last decades to include the use of bacteriocins or the producer organisms as natural food preservatives (Savadogo et al., 2009).

The safety of LAB has been evaluated by different researchers to limited extent in human beings. Lactobacillemia, antibiotic resistance, and possible production of biogenic amines in fermented products could generate undesirable adverse effects. These adverse effects rarely occur. In conclusion LAB is considered as safe (Bernardeau et al., 2008). The fundamental reasons for the development and acceptance of fermented foods can be variably ascribed to preservation, improved nutritional properties, better flavour/aroma, upgrading of substrates to higher value products and improved health aspects. (Sanders, 1999)

The genera most commonly used in industrial processes are Lactococcus, Lactobacillus, Leuconostoc, Pediococcus, Oenococcus and Streptococcus. LAB are normally used to ferment milk, meat, vegetables, cereals and wine and of recent there is keen interest in health promoting effects of LAB. Under the US Food and Administration (FDA) guidelines, LAB is generally regarded as safe (GRAS). Implying that they have been proven to be safe for human consumption through scientific procedures or through experience based on common use in food, as based on a substantial history of consumption by a significant number of individuals. (Fuller, 1989). Application of lactic acid bacteria in health the tenet, “Let food be thy medicine and medicine be thy food” was embraced ~2500 years ago by Hippocrates, the father of medicine. However, this “food as medicine” philosophy fell in relative obscurity in the 19th century with the advent of modern drug therapy. (Metchnikoff, 1908) proposed that the acid-producing organisms (lactic acid bacteria) in fermented dairy products could prevent fouling in the large intestine and thus lead to a prolongation of the life span of consumers. Later on the concept of probiotics was introduced and defined as live microbial food supplements, which benefit the health of consumers by maintaining, or improving their intestinal microbial balance. (Kolida and (Gibson, 2011). (FAO/WHO, 2002) definition which states that, “Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host” is the most acceptable at present.

LAB culture can transform a malodorous, anaerobic livestock pen, for example, into an odorless system when used in conjunction with an IMO-inoculated deep litter system (Chantsavang et al., 1993). LAB culture can also be given to most livestock species to consume through their feed and/or water as a probiotic to help foster a healthy gut flora, enhance their immune systems, and aid in digestion (Corcioni voschi et al., 2010)

The greatest potential application of LAB in human health relies on its ability to ameliorate immunologic diseases such as allergy, cancer, inflammatory bowel diseases (IBD) such as Chron’s disease and ulcerative colitis. In addition, several scientific reports indicate that regular intake of probiotics can restore/maintain microbial balance in the gastrointestinal, uro-genital and respiratory tract which is important in preventing colonization of these niches when inhabited by pathogenic bacteria. Biotransformation of deleterious compounds in the Gastro Intestinal Tract (GIT) to less deleterious compounds and production of other useful compounds such as vitamins and short chain fatty acids (SCFA) by LAB have been reported to lead to improved health. The strong inhibitory effect against the growth and toxin production of most other bacteria was suggested as one of the ways. (Farjardo et al., 2012).

LAB as probiotics may contribute a lot of ease in therapy of these infections (Mijac et al., 2006). Lactobacillus casei and Lactobacillus acidophilus possess good antifungal properties and are able to protect the immunocompromised people from opportunistic infections by Candida albicans as described by many researchers in their works in their work (Wagner et al., 2000; Polishchuk et al., 1999). LAB show antifungal activity by possessing antifungal agents against Candida albicans as described by (Anokhina et al., 2007). From above discussion it is obvious that LAB have pronounced property as anti-fungal agent.

The aim of this present work was to: 1. Isolate and characterize some of the LAB known to be human and environmental friendly 2.Discuss the positive impact of LAB in health maintenance and disease prevention.

MATERIALS AND METHOD

Sample Collection

Milk and milk products that were used for this research
were purchased locally at Bodija and Ojoor market of Ibadan Oyo State. These samples (Yorghurt {curd}, Local cheese (wara), goat milk and cow milk) were collected with a sterile McCartney bottle and transported to Microbiology laboratory of University of Ibadan in a hygienic condition for immediate use, though sample like the cow and goat milk were allowed to undergo fermentation (left in refrigerator temperature for 3-4 days) before use.

**Culture Media**

MRS (de man, Ragossa Sharpe) broth and agar medium, Nutrient agar and Peptone water were weighed and homogenized according to the manufacturer’s instructions, sterilization was carried out at 121°C for 15 minutes and was cooled to the temperature of 45°C before use.

**Isolation of microorganisms**

Serial dilutions were made from the samples using a sterile pipette. This was done by mixing 10ml of the sample to 90ml of sterile peptone water from various dilutions to give 1:10 dilution, the dilution was made up to 10^6. Using sterile pipette 1ml of appropriate dilutions was plated out using different culture media (Harrigan and McCane, 1966). After solidifying, the plates were inoculated and thereafter incubated microaerophically at 37°C for 48 hours in an inverted manner. Nutrient agar was used to resuscitate the indicator organisms, which were incubated aerobically at 30°C - 37°C for 24 hours. At the end of the incubation, representative colonies of the various isolates were selected at random and sub cultured repeatedly to obtain a pure culture.

**Culture Preservation**

Pure cultures of LAB isolates were sub cultured into maintenance medium consisting of MRS broth with 12% (v/v) glycerol and incubated at 30°C until growth becomes visible. The stock cultures were stored at 4°C for subsequent use and sub cultured at 4 weeks interval.

**Characterization of isolates**

Characterization of isolates was carried out by employing macroscopic, microscopic and biochemical tests using API kit. One milliliter of broth cultures of each isolate was used for all the tests except where otherwise stated.

**Macroscopic Examination**

The isolates were examined for growth type, shape, elevation, size, pigmentation and consistency.

**Microscopic Examination**

**Gram’s staining**

The pure isolate was stained as described by Norris and Ribbond (1971). A thin smear of the isolate was made on a clean glass slide and heat-fixed by flaming. Two drops of crystal violet are added to the smear for 1 minute. The drops of crystal was washed with water and stained with Gram's iodine solution for 1 minute. The stain was decolorized by flooding the slide with alcohol until no more violet coloration was observed. Two drops of Safranin reagent was added for 10 seconds rinsed again with tap water and blotted dry using a filter paper. Observation was made using oil immersion objective Gram-positive bacteria were characterized by purple coloration while gram-negative cells were pinkish in colour. This staining technique also shows the different shapes and arrangement of the bacteria cells.

**Biochemical Test**

**Catalase test**

MRS agar plates containing the streaked bacterial isolates and Yeast extract agar plates were streaked, yeast isolates were each incubated for 18 hours. A drop of freshly prepared 3% hydrogen peroxide was added to each plate (Seely and Vanndemark, 1972). Evolution of frothy white gas indicates a catalase positive reaction. Absence of froth indicates a negative reaction.

**Motility Test**

The organisms were grown in MRS broth for 18h at 30°C. After incubation, few drops of broth were put on a dry glass slide and examined under the oil immersion lens of the microscope. Motile cells were seen to move about randomly (Seely and Vanndemark, 1972).

**Oxidase Test**

A drop of 48 hours old culture of the isolates was placed on Whatman number one filter paper that was soaked with the oxidase reagents (1% aqueous tetramethyl-p-phenlene diamine hydrogen chloride). Formation of a deep coloration indicated a negative reaction..(Mcfadden, 2000)

**Spore stain**

Smears of the pure cultures were prepared on clean glass slides. The smears were covered with Malachite green reagent and heated until boiling and then left for 5 minutes to cool. The slide was rinsed carefully under slow-running water tap and was counter-stained with safranine for 1 minute; it was rinsed carefully and observed under the microscope. (Fall, 2011)

**Production of ammonia from arginine**

The production of ammonia from arginine was tested using the method of (Harrigan and Mc Cane 1966). A modified
MRS broth (MRS – arginine broth) without glucose and meat extract, but containing 0.3% arginine, 0.2% sodium citrate instead of ammonium citrate was used. The MRS broth without arginine was used as a control medium. 18 h old cultures were inoculated into 10ml of each broth in a test tube and incubated at 3°C for 5 days. The test samples of the culture medium after growth were placed on a spot plate to which Nessler’s reagent had been applied. Cultures producing yellow or orange colour as compared to that produced by a similarly inoculated control medium indicated the production of ammonia from arginine.

**Casein hydrolysis**

Skim milk agar prepared by adding 1% (w/v) skim milk to nutrient agar (Harrigan and McCane, 1966) was used. The agar was sterilized by autoclaving at 110°C for 10 minutes. On cooling, the medium was dispensed into sterile petri-plates and then left to solidify. The plates were then streaked across once with the isolates and then incubated for days at 30°C. Uninoculated plates served as control. At the end of incubation a clear zone around the line of streaking indicated casein hydrolysis while the absence of a clear zone indicated negative result.

**Gelatin hydrolysis**

Nine millilitres (9mls) of 10% gelatin broth (Harrigan and McCane, 1966) were dispensed into screw cap tubes and sterilized by autoclaving at 121°C for 10 minutes. The test organisms were inoculated into the tubes and incubated for 7 days. Gelatin hydrolysis was tested for by cooling the test tubes in a freezer for 15 minutes after which 10 ml of mercuric chloride solution was added. A positive reaction was indicated in tubes in which the gelatin remained in the liquid state without acidification.

**Voges – proskauer test**

Cultures were grown in glucose phosphate peptone broth for 5 days at 30°C. After incubation, 1ml of 6% 8-naphthol solution and 1ml of 10% sodium hydroxide (NaOH) was added. This is to know whether the organism after producing acid from glucose is capable of producing acetyl methyl carbinoil from the acid. The development of a pink colouration within 5 minutes is indicated as positive. (Mcfadden, 1980)

**Hydrogen sulphide production**

Lead acetate agar was dispensed into McCartney bottles and autoclaved at 110°C for 10 minutes. It was allowed to cool upright. Agar deeps were then stabbed with 18 h old cultures and incubated at 30°C for 48 h. Observation of black coloration along the line of stab indicated a positive result (Harrigan and McCane, 1966).

**Starch hydrolysis**

Equimolar amount of soluble starch was prepared and added to MRS agar without glucose or meat extract to give a 1% soluble starch before being poured to set in sterile plates. Single streaks of cultures were made on the dried plates before being incubated at 30°C for 48 hours. The plates were flooded with Gram’s iodine after incubation. Unhydrolysed starch formed a blue colouration with iodine. Clear zones around the region of growth indicated starch hydrolysis by the culture (Seely and Vann demark 1972).

**Indole test**

Each isolate inoculated into peptone-water medium and incubated at 37°C for 48 h. Five to six drops of Kovac’s reagent were then added to the culture. Development of rose pink colour on the surface of the medium indicated a positive reaction of indole production while no change in colour indicates a negative result. (Miller and Wright 1982)

**Methyl Red Test**

Glucose phosphate peptone broth was prepared as described by Harrigan and McCane (1966). Ten millilitres of the broth was dispensed into screw cap tubes and sterilized. Inoculation with test organisms was subsequently done and incubated at 30°C for 2-5 days. After incubation, a few drops of methyl red indicator were added to the culture and a resultant definite red coloration was considered positive.

**Nitrate reduction test**

Nitrate peptone water consisting of peptone water and 0.1% potassium nitrate was used. Five millilitre portions of the medium were distributed into each screw-capped test tube. Each tube contained an inverted Durham tube. The test tubes and their contents were sterilized (121°C for 15 mins) and allowed to cool before inoculating with the isolates. Uninoculated tubes served as control. The tubes were incubated at 30°C for 4 days. The ability of the isolates to reduce nitrate to nitrite, ammonia or free nitrogen was determined by adding to each tube 0.5ml of 0.6% dimethyl-naphthylamine in 5.0 ml acetic acid. The development of a red colouration indicated a positive result and the presence of gas in the Durham tube indicated the production of nitrogen gas (Payne, 1973).

**PHYSIOLOGICAL TESTS**

**Growth at different temperatures**

Tubes containing 5 ml of MRS broth were inoculated with 18 h old cultures and incubated in water bath set at the 15°C and 45°C, for 2 to 4 days (Gisbon and Abdel Malek, 1945).
**Growth at different pH**

Each bacterial isolate was streaked on MRS agar plates adjusted to pH 3.9 and 9.4. The inoculated plates were incubated for 48 h and growth along line of streak was considered positive.

**Growth at 4%, 6% and 8% NaCl**

MRS broth containing 4%, 6% and 8% sodium chloride was dispensed into screw cap tubes and sterilized. Isolates were inoculated into the medium in the tubes and incubated at 30°C for 3-4 days. Increased turbidity of medium was recorded as positive for growth. Uninoculated tubes served as control (Schillinger and Lucke, 1987).

**Homofermentative and Heterofermentative Test**

The semi-solid medium of Gibson and Abdel-Malek (1945) as modified by Stainer et al. (1964) was used. Twenty millilitres of the medium were dispensed into MacCartney bottle and sterilized. The test organisms were each inoculated in duplicate and sterile agar seal poured onto the surface of each bottle. Production of gas was indicated by gas bubbles or by the forcing of the agar seal up the tubes. Uninoculated bottles served as control.

**Sugar fermentation test**

API 50CH strip is a standardized system associating 50 biochemical tests which is used for the study of the carbohydrate metabolism of microorganism. The fermentation tests are inoculated with API 50CHL medium which rehydrates the substrates present in the strip's microtubes. Anaerobiosis in the inoculated strips was obtained by overlaying with sterile paraffin oil and incubated at 30°C and the result was read after incubation for 1-3 days, fermentation revealed by the color changes in the tubes, caused by anaerobic production of acid and detected by the pH indicator present in the chosen medium. The first tube which does not contain any active ingredient is used as a negative control. Identification was done using the identification chart for the kit.

**Identification of Isolates**

Lactic acid bacteria were initially differentiated based on colonial morphology, grams staining, catalase reaction and spore staining. The ability of the isolates to ferment carbohydrates was studied using the API 50 CH (Biomerieux) system and it was used to differentiate the isolates in to strains level.

**Identification of Lactic Acid Bacteria using API 50 CH strips and medium.**

The rapid identification of different strains of LAB isolated was done using the API 50 CH (Biomerieux) which is a standardized system, associating 50 biochemical tests for the study of the carbohydrate metabolism of microorganisms. The kit contains 10 incubation boxes (tray and lid), 10 API 50 CH strips, 10 API 50 CH medium, identification table and result sheets. API 50 CH is used in conjunction with API 50 CHL medium. One API 50 CH strip consists of 50 microtubes used to study fermentation of substrates belonging to the carbohydrate family and its derivatives (heterosides, polyalcohols, uronic acids). The holes in the incubation boxes were filled with sterile distilled water to create a humid atmosphere, the incubation tray was put on it and the strips were placed on the trays by arranging them according to the numbers on them, starting from 0-9, 10-19, 20-39, 30-39, 40-49. Pure culture of the test organisms were cultured on MRS agar plates for 18 hours and were harvested into 5ml sterile peptone water, the suspension prepared had a turbidity equivalent to 2 McFarland and it was then dispensed into the mediums for immediate use and the suspension was then drop into the strip's microtubules, the strip was labeled accordingly and incubated. The fermentation tests are inoculated with API 50 CHL medium which rehydrates the substrates present in the strip's microtubes. Anaerobiosis in the inoculated strips was obtained by overlaying with sterile paraffin oil and incubated at 30°C and the result was read after incubation for 1-3 days, fermentation revealed by the color changes in the tubes, caused by anaerobic production of acid and detected by the pH indicator present in the chosen medium. The first tube which does not contain any active ingredient is used as a negative control. Identification was done using the identification chart for the kit.

**Determination of Lactic acid, Hydrogen peroxide and Diacetyl production by LAB isolates.**

For these measurements, the test organisms were grown in MRS broth. The broths were inoculated with 0.1 ml of a suspension of LAB specie and incubated microaerophilically (Hujanen and Linko, 1996 modified). Incubation was for 72 h at 37°C. Cultures were centrifuged at 3000 rpm for 15min. Known volume of the supernatant fluid was used for all the titrations at 12 h interval except where otherwise stated.

**Quantitative Estimation of Lactic acid**

The production of lactic acid was determined by titration with 0.25 mol l⁻¹ NaOH and 1 ml of phenolphthalein indicator (0.5 % in 50 % alcohol). The titratable acidity was calculated as lactic acid (% v/v) (Fortina et al., 1990). Each millilitre of 1 N NaOH is equivalent to 90.08mg of lactic acid. The titratable acid was then calculated according to A.O.A.C. method (1995).

\[
\text{Titratable acidity} (\% \text{ Lactic acid}) = \frac{\text{Volume of sample used (ml)}}{\text{ml NaOH} \times N \text{ NaOH} \times M.E \times 100}
\]

Where:

- \(\text{ml NaOH} = \) Volume of NaOH used; 
- \(N \text{ NaOH} = \) Normality of NaOH solution; 
- \(\text{M.E} = \) Equivalence factor.
Determination of Diacetyl Formation

Diacetyl production was determined by transferring 25 ml of broth cultures of test organisms into 100 ml flasks. Seven and half millilitres of 1m Hydroxylamine solution was added to the flask and to a similar flask for residual titration. Both flasks were titrated with 0.1N HCl to a greenish yellow end point using bromophenol blue as indicator. The equivalence factor of HCl to diacetyl is 21.52 mg. The concentration of diacetyl produced was calculated according to the method of Food Chemicals Codex (1972).

\[
AK = \frac{b - s}{100E} \times W
\]

Ak = Percentage of diacetyl; b = No of ml of 0.1N HCl consumed in titration of sample; E = Equivalence factor; W = Volume of sample; S = No of ml of 0.1N HCl consumed in titration of sample.

Quantitative estimation of Hydrogen peroxide formation

Twenty ml of dilute sulphuric acid was added to 25ml of the supernatant and titration was carried out with 0.1M potassium permanganate which is equivalent to 1.7mg of hydrogen peroxide. A decolourization of the sample was regarded as the end point (A.O.A.C., 1995).

\[
\begin{align*}
H_2O_2 &+ 2KMNO_4 + 3H_2SO_4 \rightarrow K_2SO_4 + 4H_2O + O_2 \\
H_2O_2 &\rightarrow mlKMNO_4 \times NMNO_4 \times M.E \times 100
\end{align*}
\]

ml KMNO₄ = Volume of Sample used
KMNO₄ = Normality of KMNO₄
MIH₂SO₄ = Volume of H₂SO₄ used
M.E: Equivalent factor.

Survival of lactic acid bacteria at various pH levels

The method of Conway (1996) was employed. The cultures were grown in MRS broth (Oxoid) at 37°C overnight, then sub cultured into 10 ml of fresh MRS broth and incubated for another 24h. Thereafter, the cultures were centrifuged at 2000 g for 10 min at 4°C and the pellets washed twice in sterile phosphate buffered saline (PBS, pH 7.0) and resuspended in 10 ml of PBS. The concentration of the suspension was determined by comparing the turbidity with McFarland tubes of barium sulphate standard. The number of LAB per millilitre was determined by plating 10 fold serial dilutions of the suspension on MRS agar. Titre was expressed as colony forming unit per ml (cfu ml⁻¹). For each LAB strain, 0.1ml of culture suspension with a concentration of 3.7 x 10⁷ cfu/ml was added separately into a series of tubes containing 2 ml of sterile PBS at various pH values (1, 2, 3, 4 and 5). Hydrochloric acid (2M) was used to adjust the pH of the PBS as required. The tubes were incubated for 1, and 3h. The test was performed in triplicate for each selected strain. After the incubation period, 0.1 ml from each tube was cultured on MRS agar plates, incubated at 37°C for 48 h. and followed by determination of viable count.

RESULT AND DISCUSSION

A total of fifteen lactic bacteria (LAB) were isolated from fermented milk and milk products. The isolated bacteria were screened down to five strains after subjecting them to bio-chemical, physiological and morphological tests which include; Gram’s staining, catalase, oxidase, hydrogen sulphide production, nitrate reduction, ammonia production from arginine, growth in 4%, 6% and 8% sodium chloride and the use of API 50 CHL-Kit (Biomerieux france). They were identified as Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus, Streptococcus thermophilus and Lactococcus lactis, subspecies cremoris.

Frequency of occurrence of LAB strains isolated from fermented milk and milk products is shown in Table 1. Lactococcus lactis subspecies cremoris and Lactobacillus acidophilus have the highest occurrence of 40% and the least occurrence of 6.7% was observed in Streptococcus thermophilus.

All the isolated microorganisms’ colonies appeared creamy or whitish on MRS agar plates with raised edges. They were gram positive long and short rods, cocci or coccobacilli, non-motile non-endospore formers and catalase negative. Biochemical tests revealed that all LAB isolates were positive to methyl red, negative to indole test, hydrogen sulphide, gelatine hydrolysis, oxidase production and they were facultative anaerobes. Almost all the strains grew at 45°C and 15°C except few and they were non spore formers. Lactobacillus acidophilus grew at 4% NaCl, while

<table>
<thead>
<tr>
<th>ISOLATES</th>
<th>SUBSTRATE</th>
<th>NO.OF OCCURRENCE</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus brevis</td>
<td>Cow milk</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Lactobacillus plantarum &amp; fermentum</td>
<td>Goat milk</td>
<td>5</td>
<td>33.3</td>
</tr>
<tr>
<td>L.I. sp. Cremonis &amp; acidophilus</td>
<td>Local cheese (Wara)</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>Streptococcus thermophilus</td>
<td>Curd</td>
<td>1</td>
<td>6.7</td>
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<tr>
<td>TOTAL</td>
<td></td>
<td>15</td>
<td>100</td>
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</table>

Table 1. Frequency of occurrence of LAB isolated from fermented Milk and milk products
Table 2. Biochemical and Physiological Characteristics of LAB Isolated from fermented milk and milk products

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Gram Stain</th>
<th>Cellular Morphology</th>
<th>Spore Stain</th>
<th>Catalase Test</th>
<th>Oxidase Test</th>
<th>Motility Test</th>
<th>Voges-Proskauer Test</th>
<th>Starch Hydrolysis</th>
<th>Gelatin Hydrolysis</th>
<th>Methyl Red Test</th>
<th>Indole</th>
<th>H₂S Production</th>
<th>Casein Hydrolysis</th>
<th>Growth at 15°C</th>
<th>Growth at 45°C</th>
<th>Growth at 6% NaCl</th>
<th>Growth at 8% NaCl</th>
<th>Growth at 3.9 pH</th>
<th>Growth at 9.4 pH</th>
<th>Oxidative/fermentation</th>
<th>Homo/hetero fermenter</th>
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<td>S. Rod</td>
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**KEY**

HE: Heterofermenter
HO: Homofermenter
SHORT: S

Figure 1a: R-L After incubation and before incubation *Lactobacillus plantarum*

others did not grow. Most LAB strains grew at 6% NaCl while most isolates did not hydrolyze starch or casein. Negative result was recorded for nitrate reduction, gelatine hydrolysis and Voges-Proskauer tests (Table 2). The API 50CHL kit used in this work was able to differentiate the isolate to strain level. The entire LAB fermented glucose, fructose, lactose, maltose and galactose. However there is variation in fermentation of other sugars. Figure (1a&b) All the lactic acid bacteria isolated utilized glucose and this agree with the work of Knox et al. (1964)
who reported the ability of members of lactic acid bacteria to ferment glucose and other sugars such as lactose, sucrose, maltose, etc.

The quantity of Antimicrobial compound produced by LAB was investigated; it was observed that as the incubation period increases, the quantity of antimicrobial compound produced increased. However, for the quantitative estimation of Lactic Acid Bacteria, it was observed that as the incubation period increases Lactic acid concentration increased. *Lactobacillus plantarum* produces the highest quantity of antimicrobial at 72 hours of incubation (2.89 g/l) while *Lactobacillus fermentum* produced the lowest. (Figure 2).

As incubation period increases the quantity of diacetyl produced increases. *Lactococcus lactis subspecies cremoris* had the highest quantity of diacetyl at 72 hours of
incubation (1.72g/l) while *Streptococcus thermophilus* and *Lactobacillus fermentum* had the lowest quantity of 0.086g/l at the 12 hours of incubation (Figure 3).

The quantity of hydrogen peroxide produced by the test isolate varies from one species of organism to the other. Hence it was observed that as the incubation period increased the quantity of hydrogen peroxide increased up to 48 hours before declination sets in. However at 72 hours of incubation *Lactobacillus acidophilus* produced the highest concentration of hydrogen peroxide (1.91g/l) while *Streptococcus thermophilus* produced the lowest (Figure 4).

Table 4 Shows some of the alleged health benefits of consuming LAB, with significant researches to back up the claims hence LAB Enhance specific and nonspecific immune
Table 3. shows some of the alleged health benefits of consuming LAB, with significant researches to back up the claims

<table>
<thead>
<tr>
<th>LAB GRAS</th>
<th>IT’S HEALING EFFECT</th>
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<tr>
<td><strong>L. plantarum,casei, B. bifidum and S. thermophilus</strong></td>
<td>Intestinal tract health</td>
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<td>A number of studies have found LAB consumption to be useful in the treatment of many types of diarrhea, including antibiotic-associated diarrhea in adults, travellers’ diarrhea, and diarrheal diseases in young children caused by rotaviruses. The most commonly studied LAB species in these studies have been Lactobacillus plantarum, L. casei, B. bifidum and S. thermophilus. Because diarrhea is a major cause of infant death worldwide and can be incapacitating in adults, the widespread use of LAB and probiotic could be an important, non-invasive means to prevent and treat these diseases, particularly in developing countries. LAB has also been shown to preserve intestinal integrity and mediate the effects of inflammatory bowel diseases, irritable bowel syndrome, colitis, and alcoholic liver disease (Nanji et al., 1994). In addition, lactic acid bacteria may improve intestinal mobility and relieve constipation, particularly in seniors (Seki et al., 1978).</td>
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<td><strong>S. thermophilus, L. fermentum</strong></td>
<td>Cardiovascular disease</td>
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<td>There is preliminary evidence that use of probiotic lactobacilli and metabolic by-products potentially confer benefits to the heart, including prevention and therapy of various ischemic heart syndromes (Oxman et al., 2001) and lowering serum cholesterol While the Consultation believes these findings to be important, more research and particularly human studies are required before it can be ascertained that probiotics confer health benefits to the cardiovascular system. (De Roos and Katan, 2000).</td>
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<td><strong>L. acidophilus, L. paracasei</strong></td>
<td>Immune system</td>
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<td>Evidence from in vitro systems, animal models and humans suggests that LAB can enhance both the specific and nonspecific immune response, possibly by activating macrophages, increasing levels of cytokines, increasing natural killer cell activity, and/or increasing levels of immunoglobulins. In spite of limited testing in humans, these results may be particularly important to the elderly, who could benefit from an enhanced immune response. (Sanders, 1999)</td>
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<td><strong>S. thermophilus, L. bulgaricus</strong></td>
<td>Lactose intolerance</td>
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<td>Several lines of evidence show that the appropriate strains of lactic acid bacteria, such as S. thermophilus, L. bulgaricus and other lactobacilli in fermented milk products, can alleviate symptoms of lactose intolerance by providing bacterial lactase to the intestine and stomach. Because lactose intolerance affects almost 70% of the population worldwide, consumption of these products may be a good way to incorporate dairy products and their accompanying nutrients into the diets of lactose intolerant individuals. (Agerholm-Larsen, 2000)</td>
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<td><strong>L. paracasei, fermentum plantarum</strong></td>
<td>Role of lactic acid bacteria in treating ulcer</td>
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<td>Myllyluoma et al., in their study reported the beneficial effects of Lactic acid bacteria in gastric ulcer. They proposed that these effects were due to the destructive actions of lactic acid bacteria on H. pylori. If LAB is used in combination of antiulcerative therapy then results are astonishingly fast recovery and improved efficacy of therapy. In LAB use of Lactococcus rhamnosus as an adjuvant therapy during H. pylori eradication has been proved (Myllyluoma et al., 2007). Lactococcus rhamnosus not only is used as adjunct in anti-ulcerative therapy but also reduced ethanol-induced mucosal lesion. Pre-treatment with Lactococcus rhamnosus also significantly increases the basal mucosal prostaglandin E2 (PGE2) level, also attenuates the suppressive actions of ethanol on mucussecreting layer and transmucosal resistance and reduces cellular apoptosis in the gastric mucosa. Hence we can say Lactococcus rhamnosus is an antulcerative in many ways as reported by researchers (Lam et al., 2007).</td>
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<td><strong>L. acidophilus, plantarum casei, fermentum</strong></td>
<td>Cancer</td>
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| Studies of the effect of probiotic consumption on cancer appear promising. Animal and in vitro studies indicate that probiotic bacteria may reduce colon cancer risk by reducing the incidence and number of tumors. One clinical study showed an increased recurrence-free period in subjects with bladder cancer Aso and Akazan 1992. Results, however, are too preliminary to develop specific recommendations on probiotic consumption for preventing cancer in humans.Kim et al. (2006) in their study found that LAB such as Lactobacillus rhamnosus ATCC 9595 was useful in preventing colon cancer in human being. They conducted experiments on two cell lines of cancer, i.e., PANC-I (pancreas) and HI-29 (colon). They found that Lactic acid bacteria successfully decreased the cancer growth. The anticancer activity of Lactobacillus rhamnosus was might be due to the induction of apoptosis by two expolysaccaridates of bacteria name rEPS (released expolysaccharides) and chEPS (cell bound expolysaccharides). rEPS was more effective in preventing cancer than cbEPS. Uncontrolled growth of colon cells may be affected by the different strains of LAB. Different strains of LAB exhibit different action which ranges from inhibition of
response, inhibit pathogen growth and translocation, stimulate gastrointestinal immunity, reduce chance of infection from common pathogens (Salmonella, Shigella), reduce risk of certain cancers (colon, bladder! Detoxify carcinogens Lower serum cholesterol concentrations improve urogenital health to name just but a few. (Savadogo et al., 2006)

**Conclusion**

This paper shows that the quantity of antimicrobial compounds produced by these LAB strains has the potential to inhibit pathogenic and spoilage organisms. This justify the use of organic acids, hydrogen peroxide and diacetyl particularly its combination to antagonize pathogenic and spoilage organisms. Besides these organisms are added as dietary adjuncts to commercially fermented milk products and the intake of these bacteria may have beneficial effects on human health.

**REFERENCES**


Fall, (2011) Jackie Reynolds Richland College BIOL2421