European Journal of Biotechnology and Bioscience
Online ISSN: 2321-9122, Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 4; Issue 7; July 2016; Page No. 34-41

Phytic acid degradation by selected lactobacilli isolated from fruits and vegetables and their potential as probiotics

[*1, 2] Bertrand Tatsinkou Fossi, [1, 2] Che Mercy Akwanwi, [1, 2] Nchanji Gordon Takop, [1, 2] Ebenye Yvone Bille, [1, 2] Samuel Wanji

[1] Department of Microbiology and Parasitology, Faculty of Science, University of Buea, POBox 63, Buea, Cameroon
[2] Research Foundation in Tropical diseases and environment, POBox 474, Buea, Cameroon
*Corresponding author; E-mail: tatsinkoufossi@yahoo.fr

Abstract
Fruits and vegetables are important part of the diet of most humans. They are highly nutritive, and constitute an important source of minerals and vitamins. However the widely consumed fruits and vegetables are rich in phytic acid that causes reduction in the absorption and bioavailability of nutrients and minerals. The use of probiotic bacteria such as phytic acid degrading lactic acid bacteria (LAB) in the diet could help to prevent the chelation of mineral by phytates. Therefore the aim of this study was to screen, select and characterise phytate degrading LAB isolated from fruits and vegetables. LAB were isolated from 106 fruits and vegetables samples collected in Buea and Limbe using pour plating method on de Man Rogosa and Sharpe (MRS) agar. The isolates were characterised using the catalase test, Gram staining and the API 50 CHL BioMerieux kit. The phytate degrading activity of LAB was carried out qualitatively on modified MRS agar by point inoculation and the phytase activity was quantified in broth by spectrophotometric method. Thirty three LAB isolates were isolated. In which Twenty one produced a clear zone of hydrolysis on modified MRS agar demonstrating their phytate degrading potentials. *Lactobacillus rhamnosus* and *Lactobacillus plantarum* showed the highest phytate degradation both qualitatively and quantitatively, producing the highest amounts of the enzyme phytase. Both isolates also had a good tolerance to bile and acidic conditions, there by showing their ability to survive in the gastrointestinal track. This work shows a new perspective on the phytate degrading power of LAB thereby highlighting the ability of LAB to decrease malnutrition due to mineral deficiency.

Keywords: Lactic acid bacteria, Phytic acid, Antinutrient, Phytase activity, Probiotics

1. Introduction

Phytic acid also call phytate is an anti-nutrient, highly present in cereals, grains and nuts which make up most the diets of most humans and animals. But humans and animals do not contain sufficient amount of phytates in the gastrointestinal track to break down this phytate. Since animals will not break down this acid, it is excreted in the animal droppings into the environment thereby polluting the environment with phosphorus leading to eutrophication (Mittal *et al*., 2012) [16]. Meanwhile the inability of humans to digest this phytate leads to the formation of complexes with proteins and amino acids and reduce the absorption and bioavailability of essential minerals especially iron, calcium, zinc and magnesium, causing malnutrition (Lopez *et al.,* 2000; De Angelis *et al*., 2003; Reale *et al.,* 2004; Palacios *et al*., 2005) [11, 4, 17]. One of the problems in the developing countries is the inability to combat malnutrition in both children and adults. According to the United Nations World Food Programme (WFP) and the UN Food and Agriculture Organization (FAO) in 2009, 23% of Cameroonians are suffering from malnutrition (Engle *et al*., 2014) [7].This mineral deficiency leads to diseases like Iron deficient anaemia, Osteoporosis, magnesium and zinc deficiency. In Cameroon, the prevalence of deficiencies in calcium, magnesium, zinc, was 73.56%, 45.96% and 62.98%, respectively of the children between three and five years of age. The prevalence of iron deficiency (ID) ranged from 14.2 to 68.4% among children (3-5yrs), and 12.0 to 47.4% among children greater than 5yrs (Nolla *et al*., 2014) [19]. This prevalence of mineral deficiency in children is due to their low intake of meat, fresh fish, vegetables and fruits and high intake of rice and beans which have high phytate content. Since microbial enzymatic degradation of phytate is the best method of phytate degradation. The use of probiotic LAB (Generally regarded as Safe) with potential of degrading phytate is novel approach to reduce the malabsorption of mineral cause by phytate.

This work aims at Characterizing phytic acid degrading LAB isolated from fruits and vegetables and their potential as probiotic.

2. Materials and methods

Study area and design

This study was carried out with samples from Buea and Limbe municipalities in the Fako Division in the South West Region of Cameroon bounded to the North by tropical forest on the slopes of Mont Cameroon (4100m above sea level). The Mountain range extends to the beautiful sandy beaches of the Atlantic Ocean. Our study was based on the collection of already rotten fruits and vegetables and used as a potential source of probiotics lactic acid bacteria. So we selected pineapples and Oranges as the fruits of choice, Carrots, Cabbages and Tomatoes are the vegetables and some soil samples where used in this study.

Our study was a Randomized controlled trial in which we sought to screen and select the different LAB strains in various fruits and vegetables for their ability to produce the enzyme phytase enabling it to hydrolyse phytic acid which chelate certain important nutrients in the body.

Sample collection and processing

A total of 106 samples were collected from two municipalities in the South west Region of Cameroon. Fifty five (55) samples were collected from Buea and fifty one (51) from Limbe. Already rotten oranges, pineapples, cabbages, carrots and tomatoes and five soil samples each were collected from local markets and local vendors in Buea and Limbe municipalities. The samples in this study were collected in to zip lock bags, stored in a flask with Silicon gel and transferred to the Lab. The samples were kept refrigerated at 4°C in the ziplock bag until the beginning of analysis. These samples were analysed and characterized at the Research Foundation in Tropical Diseases and Environment (REFOTDE), Buea, Cameroon.

The fruits and vegetables were peeled with a sterilised knife and crushed with a mortar and pestle (sterilised 70% alcohol and flamed) to extract the juice. 1ml of the juice was homogenized in 9ml peptone water before serial diluting.

Media preparation

In a disinfected environment (using bleach disinfectant and tissue paper) and using a cleaned spatula, given masses of MRS (de Man, Rogosa, and Sharpe) broth and agar, and peptone water, obtained from LIOFILCHEM DIAGNOSTICI were weight to prepare medium according to manufactures instruction for culturing.(de MAN, 1960) [5].

Isolation of phytic acid degrading lactic bacteria

All our processed samples were serially diluted in a 1:10 dilution using peptone water. The liquid samples were thoroughly mixed by inversion before dilution in which 1 ml of sample was pipette into 9 ml of peptone water. Dilutions were repeated up to a 10^4 dilution. Using the pour plate method, 0.1 ml from each dilution was inoculated on Petri dishes and 15 ml of MRS agar was added into each of these Petri dishes swirled and allowed to solidify. After solidification, the plates were incubated at 37°C for 48 hours. After incubation colonies that were catalase negative and Gram positive, were sub-cultured to obtain pure colonies and stored at 4°C until further analyses- Carbohydrate fermentation patterns of LAB were determined using API 50 CHL kit (BioMerieux, France). The APILAB PLUS database software was used to interpret the results.

Degradation of phytic acid by lactic acid bacteria
Extraction of Cell Free Supernatant

Isolates were sub-cultured in MRS broth and incubated at 37°C until the stationary phase of growth was attained (18–24 h). Cells were harvested by centrifugation (8000 rpm for 15 min at4 °C) and washed with 50mM Tris–HCl (pH 6.5). The cell pellet (10^7–10^8 CFU/ml) thus obtained was suspended in saline (0.85%) and in 100mM sodium acetate–acetic acid buffer (pH 5.5). The saline suspension was used for plate assay method to test for the phytate degrading ability, whereas buffer suspension was used for enzyme activity and biochemical assay.

LAB degrading ability of phytic acid on Agar

Phytic acid degradation ability was screened using modified MRS (mMRS) agar (MRS agar with 0.2% calcium chloride, and 0.25% sodium phytate as the major source of phosphate). The media was prepared by dissolving MRS agar in distilled water and autoclaving at 121°C for 15min. After autoclaving, the latter two salts were dissolved in distilled water; filter sterilized using a 0.22µm filter and added to the autoclaved MRS medium at 55°C before pouring in agar plates. Three (3) µL of the saline suspension was pipette and point inoculated on the surface of the modified MRS agar. The plates were then incubate at 37°C for 24hrs. After incubation, the colonies where washed from the agar surface using distilled water. The plates were stained with 2% cobalt chloride and incubated for five minutes at room temperature and counter stained with 6.25% ammonium molybdate for five minutes at room temperature and the plate now observed for zone of phytate hydrolysis.

Phytase assay

Phytase activity was assayed by measuring the amount of liberated inorganic phosphate from sodium phytate (Nielsen *et al*., 2008). One unit of phytase activity (U) was defined as the amount of enzyme that produces 1 umol of inorganic phosphorous per min at 50 °C. This enzyme activity was determined by incubating a mixture of 50 µL of buffered cell suspension with 50 µL of 2 mM sodium phytate prepared in 100 mM sodium acetate buffer (pH 5.5) at 37 °C for 30 min. The reaction was then stopped by adding 100 µl of 10% (w/v) trichloroacetic acid solution (TCA). Blank was prepared by adding 10% TCA solution to the cell supernatant before the substrate was added. The absorbance was read at 0min, and after 30min and the inorganic phosphorous released was quantified using the Spectrophotometer at 630 nm to be able to calculate the concentration of phytase in the cell supernatant. (Nielsen *et al*., 2008) [18].

Some characteristics features of phytate degrading LAB
Tolerance to Acidic conditions.

LAB isolates were cultured in MRS broth, for 18 hours at 37 °C. The cells were harvest by centrifuging for 10 minutes at 5000 rpm at room temperature. The cell pellets were then suspended in peptone water (standardized to McFarland 2). Ten percent (10%) of the suspension was inoculated into 10 ml MRS broth were the pH had been adjusted to 1.5, 3.5 and 7.0 with 0.1 N HCl. The culture was incubated at 37 °C for 1, 2, 3 and 4 hr and at every 1 h interval, the optical density (O.D.) at 630 nm using UV–visible spectrophotometer was recorded and incubated again for 24hrs and the optical density was recorded a second time. Growth leading to increase in the O.D. was considered as the tolerance ability of the LAB to acid.

Bile Tolerance

LAB isolates were cultured in MRS broth, for 18 hours at 37 o C. The cells were harvest by centrifuging for 10 minutes at 5000 rpm at room temperature. The cell pellets were then suspended in peptone water (standardized to McFarland 2). Ten percent (10%) of the suspension was inoculated into 10 ml MRS broth containing 0.15 % (w/v) oxgall-bile, 0.30 % (w/v) oxgall-bile and one set of MRS broth without oxgall-bile to serve as the control. The culture was incubated at 37 °C for 1, 2, 3 and 4 hr and at every 1 h interval, the optical density (O.D.) at 630 nm using UV–visible spectrophotometer was recorded and incubated again for 24hrs and the optical density was recorded a second time. Growth leading to increase in the

O.D. was considered as the tolerance ability of the LAB to acid.

Statistical analysis

Excel 2007 will be used to obtain general statistics parameters. The statistical software package, Statistical Package for Social Scientist (SPSS) version 17.0 will be uses to examine differences in means of phytase produced by each LAB isolate.

3. Results

Isolation and phenotypic Characterization of LAB

The identification of lactic acid bacteria was achieved by first culturing on MRS agar which is the selective media of choice for the growth of LAB. This was preceded with different biochemical tests: catalase test and Gram staining. Table 1 presents the results of the biochemical test performed to screen and identify the various LAB isolates. Isolates which were catalase negative and Gram positive rods were considered presumptive LAB isolates

Table 1: Preliminary Characteristics of lactic acid bacteria Isolates

Sample site	Isolate	Catalase reaction	Gram reaction
Town	AT2, CP2	_	+
MILE 16	CP1, CO1	−	+
MUEA	MC1, MO1, MC3	_	+
OLD TOWN	OO1, OT5, OCb6, OC2, OC5, OO2, OCb3, OC2, OCb4, OO4, OP1	−	+
NEW	NS, NCb3, NT1, NC2, NCb2	−	+
STADIUM	SP4, SP2	−	+
HOCKER	HP1, HP2	−	+

+ signifies isolate that were Gram positive: - signifies isolates that where Catalase negative: O= Orange; P= Pineapple; C=Carrot; T=Tomatoes; Cb=Cabbage S= Soil.

Phytic acid degradation by lactic acid bacteria on modified MRS agar: Thirty Three isolates from different sources (Oranges, Pineapples, Carrots, Tomatoes and Cabbages) were screened for their phytate degrading ability using the modified MRS (mMRS) agar. Twenty one (21) of these isolates produced a clear halo of phytate hydrolysis on agar indicating phytate degradation by the isolates. Table 2 shows the various isolates that produced a halo on mMRS agar and the source of the isolates.

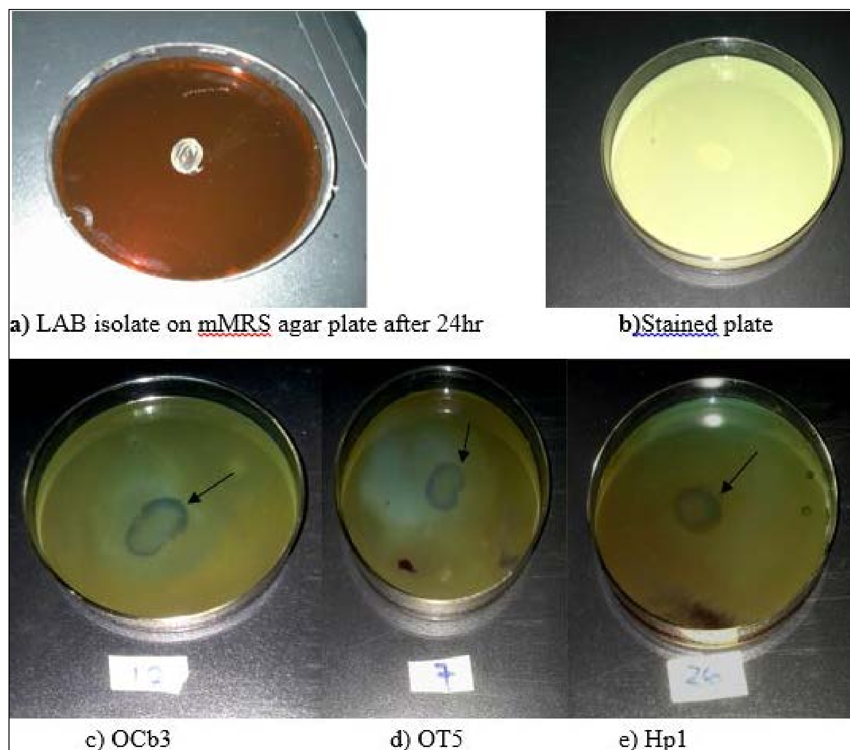

Fig 1: Plate assays for phytate degradation by LAB Phytase activity is materialized by the formation clear zone of hydrolysis around the inoculating wells.

Fig. 1 a and b show plates of LAB isolate after incubating for 24 h and after staining with cobalt chloride and ammonium molybdate respectively. After allowing plates for a few hours, Fig. 1 c, d and e shows the zone of hydrolysis by three different LAB (OCb3, OT5 and Hp1 respectively) isolates on phytate. Clear Halo of phytate hydrolysis produced by three LAB isolates were observed after incubating plates of LAB at 37°C for 24hrs by point inoculating on agar, washing colonies off the agar surface, and staining with 2% cobalt chloride and counter staining with 6.25% ammonium molybdate.

Table 2: Phytate degrading activity of LAB isolates

Source of LAB	Isolate	Hydrolysis
Pineapple	Hp1,OP1,CP1,Hp2,Sp4,CP2,Pi2	+
Orange	MO1,OO1,OO4,CO1	+
Tomatoe	OT5,NT1,AT2	+
Cabbage	NCb3,OCb6,OCb3	+
Carrot	OC2,OC5,MC1,NC2	+
Rafia	Ra2	+
Soil	NS	+

+ signifies isolates that produced zone of hydrolysis on agar.

Phytic acid degradation using LAB broth

All the selected isolates were able to produce phytase in broth, the isolates MO1 and HP2 exhibited the highest phytase activity. Phytase production reach 18.1±0.1 and 16.8±0.3 respectively for the isolates MO1 and MO2 (Table 3)

Table 3: Phytase activity exhibited by each isolate in the fermenting broth

Strains	Phytase activity (U/ ml)
Mould	16.2±0.2
NT1	11.2±0.3
OP1	13.3±0.9
MO1	18.1±0.1
MC1	4.7±0.2
AT2	5.9±0.02
OCb6	4.4±0.04
HP1	12.4±2
HP2	16.8±0.3
0C2	3.9±0.19
OT5	14±1
Ra2	4.2±0.11
NC2	4.5±0.4
CP2	2±0.3
NC4	3.2±0.3
OC5	2.8±0.22
NCb3	3.7±0.5
Cb3	5.1±0.06
SP4	3.1±0.2

Other properties of selected LAB

The isolates that produced the highest amount of phytase were further characterised using the API 50 CHL kit. The results are shown in Table 4, after 48 hours of incubation. The obtained biochemical profile of the LAB strains identified the isolates; Isolate MO1 = *Lactobacillus rhamnosus,* Isolate HP2 =*Lactobacillus plantarum,* Isolate OT5=*Lactobacillus rhamnosus*, Isolate HP1= *Lactobacillus plantarum 1*, Isolate NT1 = *Lactobacillus pentosus*,

Tolerance to acid

The ability of selected LAB isolate to survive in acidic conditions is shown in Fig.2. All the two isolates *L. plantarum* and *L. rhamnosus* did not survive nor grow in the acidic condition of pH 1.5. *L. plantarum* and *L. rhamnosus* could tolerate the acidic conditions of pH 3.5 for all the 4 hours. Both isolates survived and grew at the pH 7.0 after 4hrs of incubation. The results presented in the Figures below were obtained after 24 hours incubation of the LAB strains at 37°C in the acidic conditions. These were obtained after culturing them at 37°C for the allocated times of 0, 1, 2, 3 and 4 hours.

Tolerance to bile

Figures 3, presents the survival trend, after 24 hours incubation at 37°C of the LAB strains in MRS broth with and without bile. This was obtained after culturing at 37°C for the allocated times of 0, 1, 2, 3 and 4 hours. All the LAB isolates survived one to four hours incubation in MRS broth containing 0.15 %(w/v) and 0.30 %(w/v) bile with higher survival rates in 0.30 %(w/v) concentration than in 0.15 %(w/v), compared to the control without bile.

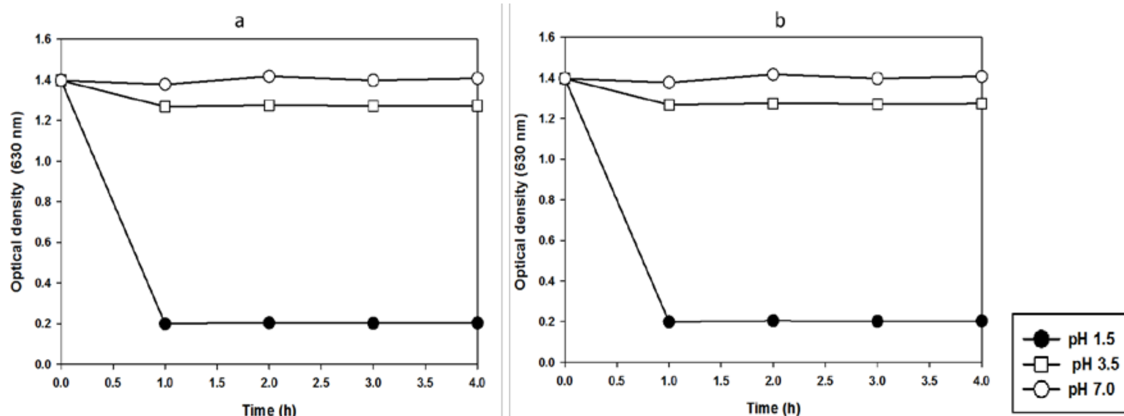

Fig 2: Survival of *Lactobacillus rhamnosus* (a) and *Lactobacillus plantarum* (b) in acid

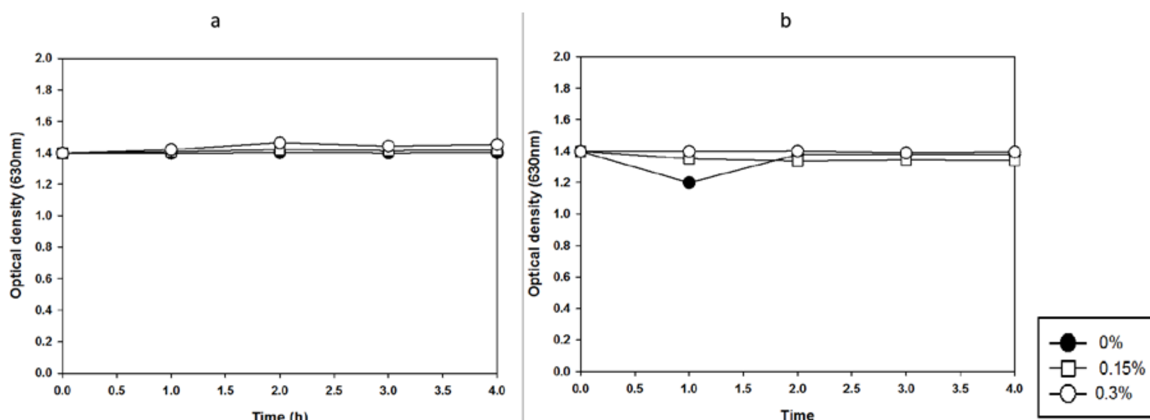

Fig 3: Survival of Lactobacillus rhamnosus MO1 (a) and Lactobacillus plantarum (b) HP2 in bile

Table 4: Biochemical characterization of LAB using API 50 CHL Bio Merieux kit

Test number	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Strains code	control	Glycerol	Erythritol	D-arabinose	L-arabinose	Ribose	D-xylose	L-xylose	Adonitol	ß methyl-D-Xyloside	Galactose	Glucose	Fructose	Mannose	Sorbose	Rhamnose	Dulcitol	Inositol	Mannitol	Sorbitol	Methyl-D-mannoside	Methyl-D-glucoside	N-Acetyl-Glucosamine	Amygdalin	Arbutin	Esculin	Salicin	Cellobiose
MO1	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
HP2	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+
OT5	-	-	-	-	+	+	-	-	-	-	+	+	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+	+
HP1	-	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	-	+	?	+	+	+	+	+	+
NT1	-	-	-	-	?	+	+	-	-	-	+	+	+	+	-	-	+	-	+	+	-	-	+	-	+	+	+	+

Test number	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	
Strains code	Maltose	Lactose	Melibiose	Sucrose	Trehalose	Inulin	Melezitose	Raffinose	Starch	Glycogen	Xylitol	ß Gentiobiose	D-turanose	D-lyxose	D-tagatose	D-fucose	L-fucose	D-arabitol	L-arabitol	Gluconate	2-Keto-Gluconate	5-Keto-Gluconate	Identified species
MO1	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	-	-	-	-	?	-	-	*Lactobacillus rhamnosus*
HP2	+	+	+	+	+	-	+	+	-	-	-	+	+	-	+	-	-	?	-	?	?	?	*Lactobacillus plantarum*
OT5	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	?	-	-	*Lactobacillus rhamnosus*
HP1	+	+	+	+	+	-	+	+	-	-	-	+	+	-	-	-	-	?	-	?	?	?	*Lactobacillus plantarum*
NT1	+	-	-	+	+	+	+	-	-	-	-	-	+	-	+	-	-	-	-	?	-	?	*Lactobacillus pentosus*

+ positive reaction; -negative reaction; ? non conclusive

4. Discussion

Fruits and vegetables contain high amount of minerals and vitamins. They are perishable commodities and also contain anti-nutrients like phytate. Therefore, there is a pressing need for fruit and vegetable grade LAB to be utilized in their fermentation processes to increase their shelf life and to promote their nutritional benefits or nutraceutical supplements (Famularo *et al*., 2005) [8]. Several clinical studies have elucidated that the phytate content of certain foods such as whole wheat products, cereals and grains is a foremost determinant negatively governing the nutritional balance of trace minerals and proteins in subjects on a regular vegetarian diet (De Angelis *et al*., 2003) [4]. This leads us to why we are conducting this study which deals with the role of LAB in degrading this phytate. Lactic acid bacteria (LAB) can be isolated from a variety of foods. It is the main organism responsible for fermentation in most of these foods. Lactic acid bacteria are found in dairy products, fermented meat, sour dough, fermented vegetables, fruits, silage beverages-including wine, on plants, in sewage (Azadnia and Khan, 2009) [1]. In our study, LAB were isolated from fruits and vegetables collected from markets in the Buea and limbe municipalities. We used two different areas so as to vary the strains of LAB isolated since both areas have different climatic conditions. Growth on de Man, Rogosa and Sharpe (MRS) agar coupled with catalase test and Gram staining are characteristic methods of isolating LAB. Earlier microbial investigations of fermented fruits and vegetables (Pasquale *et al*., 2014) [22] have reported the predominance and importance of LAB in the fermentation process. Our studies confirmed the predominance of LAB and we isolated 33 presumptive LAB isolates. The presences of LAB are assumed to be responsible for the relatively high lactic and acetic acid production and thereby outcompeting non-acid-tolerant species. Isolates that produced the highest amount of enzyme phytase in our study were further identified by biochemical characterisation using the API 50 CHL kit (API system, BioMérieux, France). This technique is widely used for the identification of lactic acid bacteria. This method is simple, and involves the fermentation of different carbohydrates to acids which causes a decrease in pH, after 48 hours of incubation (Conter *et al*., 2005) [3]. The decrease in pH is shown by a change in colour of the indicator, from purple to yellow with the exception of the esculin test (tube 25), that changes from purple to black. Five LAB isolates were identified; one isolate from Orange, two from pineapple and the remaining two from tomatoes. These isolates were identified as follows: Isolate MO1 = *Lactobacillus rhamnosus,* Isolate HP2 =*Lactobacillus plantarum,* Isolate OT5=*Lactobacillus rhamnosus*, Isolate HP1= *Lactobacillus plantarum 1*, Isolate NT1 = *Lactobacillus pentosus*. This study is similar to a study carried out by Suk Hee *et al.,* (2012) [24] who also successfully isolated and identified similar LAB strains from kimchi using the API 50CHL kit. LAB could be successfully isolated from fruit and vegetables because they contain specific nutrients such as vitamins, minerals, and their acidic nature provides conducible medium for fermentation by LAB

Phytases are enzymes that have successfully been used in degrading phytate. There are many different sources of this enzyme but microbes have been exploited on their phytase activity and their ability to degrade phytic acid and produced positive results. Several microbes like, *Bacillus* (Nabil *et al*., 2013) [17], *Escherichia coli* and *Klebsiella terrigena* (Greiner *et al*. 1997) [10]. has been used in several other studies to investigate their phytate degrading potential and they all showed complete degradation of phytate Although these microorganisms have proven to be very effective, the use of most of these microorganisms has proven to be less effective because they are pathogenic organism producing toxic substances that are detrimental to the health of animals and humans. This promotes the need of our study to use probiotic microorganism to degrade this phytic acid there by decreasing its activity in the gastrointestinal track. Lactic acid bacteria produce many antimicrobial agents, the ability of LAB to produce the enzyme phytase make them suitable for investigating their phytate degrading activity *in vitro* and in food stuff. Phytate-degrading activity has been reported in some *Lactobacillus* species and has been suggested to improve the nutritional quality of fermented cereal grains and De Angelis *et al*., (2003) [4] further reported the purification of a phytase from *Lactobacillus sanfranciscensis* thereby boosting the need for other strains to be investigated for the ability of produce phytase. In contrast to the partial PA degradation achieved by application of selected starter cultures, the addition of purified *A. niger* phytases (Yetti *et al*., 2010) [25], showed enzyme production with complete PA degradation, but it is not recommended for use due to its ability to produce toxic substances, we thereby used them in our study as positive controls.

Since certain LAB has previously been reported to be capable of degrading PA we have thus screened 33 presumptive LAB isolated from fruits and vegetables for their ability to degrade phytate. Twenty one PA degrading isolates showed phytate degradation. The degradation of phytate by LAB was carried out on agar medium by the point inoculation method and in broth using turbidometric method. The phytate degradation on agar gives a qualitative degrading power of the different LAB isolates while the degradation in broth gives a quantitative degrading activity of the different LAB isolates. For the qualitative method on agar, modified MRS agar that is agar containing phytate was used for detection and qualitative evaluation of phytate degradation. Phytase activity was indicated by zones of clearing after culture on agar. However, reduced pH around colonies of acid-producing bacteria may also cause the appearance of zones of clearing. False-positive detection of phytase activity was reduced by staining with aqueous cobalt chloride and ammonium molybdovanadate solutions. The different LAB isolates revealed varied degrading activity on agar with degradation highest and most significant with *Lactobacillus rhamnosus* gotten from Oranges and *Lactobacillus plantarum 1* isolated from pineapples. The varying degrading activity of LAB on phytate may be attributed to the production of the enzyme phytase, which was confirmed when the isolates degraded sodium phytate in the presence of calcium chloride producing a clear zone of hydrolysis on agar.

The quantitative degrading activity of phytate by LAB is a more specific method since it determines the actual amount of phytase produced by the isolates. *Lactobacillus plantarum* and *Lactobacillus rhamnosus* similarly to the agar method produced the highest amount of phytase that is, 18 and 16 U phytase per min as shown in Table 4. There by enabling these isolates to be used as potential sources in the production of the enzyme phytase. This implies that phytases produced by these

strain can be used as food and feed supplement in human and animal diet, also for reduction of phosphorus pollution problems in areas of livestock production. Since these isolates *Lactobacillus rhamnosus, and Lactobacillus plantarum 1* showed the highest degrading effect of phytate in both methods, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* make up the main LAB species on which phytate degradation have been based on in our study. Similar studies like Ponnala *et al*., (2009) [23] who used *L. rhamnosus* and *Lactobacillus amylovorus* isolated from chicken intestine and showed phytate degrading activity with phytase activity of 15 and 8 U which is similar to the phytase activity of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* used in our study. Futhermore, Maren *et al.,* (2014) [14] isolated *Lactobacillus plantarum* from Ethiopian tef-injera batter respectively and they showed phytate degrading activity by degrading the phytic acid content of Ethiopian tef by 71% that is decreasing the phytic acid from 1.05 g/100 g dm to 0.87 g/100 g dm. Ponnala *et al.* (2009) [23] also reported phytate degrading *Pediococcus Pentosaceus* and *Pediococcus pentosaceus* from chicken and fish intestinal origin but we were unable to isolate this strains from fruits and vegetables which is similar to our isolate NT1 identified to be *L. pentosus* which also showed degrading activity on phytate but very little has being said on the degrading activity of *L pentosus.*

Selected potent phytate degrading LAB were evaluated for some additional characteristic features like their ability to survive in the gastrointestinal track. A probiotic bacteria need to be resistant to low pH of the stomach and bile salt of the upper gastrointestinal tract. One of the main criteria for selection of a microorganism is its tolerance to acid. Resistance to pH 3.5 is often used in *in vitro* assays to determine the resistance to stomach pH (Ekundayo, 2014) [6]. Food usually stays in the stomach for 3 hours (Kavitha and Devasena, 2013) [11], and this time limit was taken into account since to reach the small intestines, probiotics have to pass through the stressful conditions of stomach (Çakır 2003) [2]. Although in the stomach, pH can be as low as 1.0, in most *in vitro* assays, pH 3.5 has been preferred due to the fact that, a significant decrease in the viability of strains is often observed at pH 1.0 (Kavitha and Devasena, 2013). In our study the screening of strains resistant to growth at low pH, that is MRS broth of pH 1.5, 3.5 and 7.0 was carried out during a period of 0 to 4 hours. Similarly, bile salt plays an important role in physiological function with respect to the survival of LAB in small intestine. Gilliland *et al.* (1984) [9] reported that 0.3% ox-bile is considered to be a crucial concentration to evaluate bile tolerant probiotic organisms for human. The bile used in our study was Oxgall which is a natural dried bovine bile component. The screening of strains resistant to growth in high bile concentrations that is MRS broth with bile concentrations of 0.15% and 0.30% was carried out during a period of 0 to 4 hours. The growth of strains in MRS broth without bile and without any pH modifications was used in our study as our control. The two strains of LAB that showed best phytase activity in our study were invested for their probiotic properties in our study. These isolates were *Lactobacillus rhamnosus* and *Lactobacillus plantarum* 1. Both isolates did not tolerate the acidic condition pH 1.5, as neither growth nor survival was observed after 4hrs as shown in figure 2 and 3. They tolerated the acid condition of pH 3.5, they had a high survival rate after the first hour, which dropped slightly and levelled off at the 4th hrs. They also tolerated the neutral condition of pH 7 after 4hrs. For their survival and growth in bile, both isolates *L. plantarum* and *L. rhamnosus* showed higher growth rates after the addition of 0.3% (w/v) Oxgall, after three hrs with a slight decrease in growth at the 4th hour. *L. rhamnosus* had slightly lower growth rates as compared to *L plantarum 1* in 0.3% bile addition after 3 hrs. The results of the analysis of MRS supplemented with 0.15% (w/v) Oxgall, indicates that *L. plantarum* had a slightly lower than normal growth rate at 0.15% than the normal MRS medium after four hour than *L rhamnosus* who maintained a high growth rate than the unsupplemented MRS medium after 4 hrs as are represented in figure 8 and 9. Both strain exhibited higher growth abilities in increasing bile concentration. These results are similar to results gotten by Maryam *et al*., (2009) [15] who also noticed a high survival and growth when *L rhamnosus* and *L plantarum 1* where incubated in MRS supplemented with 0.30% and 0.15% bile. This implies that both isolates can survive under extreme bile conditions.

We have demonstrated proof of the principle of enzyme production and degradation of phytate by LAB and the ability of these microorganisms to survive and grow in the gastrointestinal tract there by proofing their probiotic properties. Therefore, the identification of probiotic organisms producing phytase or other important enzymes may offer a value-added benefit in addition to the food safety to humans and animal health benefits traditionally associated with probiotic administration.

5. Conclusion

Probiotics is an emerging tool in the prevention and fight against infections of the human system and the problem of malnutrition in Cameroon and the world in general. Therefore, this study will help improve the nutritional and general health situation of the public. From this study, we can conclude that

- *Lactobacillus rhamnosus Lactobacillus pentosus,* and *Lactobacillus plantarum 1*, can be isolated from fruits and vegetables collected from markets in the Buea and Limbe municipalities in the South West Region.
- Two isolates *Lactobacillus rhamnosus* and *Lactobacillus plantarum* from our fruits and vegetables produced the highest levels of phytic acid degrading on sodium phytate producing high amount of phytase activity.

Both phytate degrading isolates *Lactobacillus rhamnosus* and *Lactobacillus plantarum* were tolerant to bile even after 4hrs and to acid at pH 3.5 enabling them to survive in the gastrointestinal track of humans.

6. Acknowledgments

The authors would like to acknowledge the Microbiology Unit of REFOTDE (Research Foundation for Tropical Diseases and Environment), Buea, Cameroon and the Biotechnology Unit of University of Buea, Cameroon, for providing facilities for research.

7. References

1. Azadnia P, Khan Nazer AH. Identification of lactic acid bacteria isolated from traditional drinking yoghurt in tribes of Fars province. Iranian Journal of Veterinary Research. 2009; 10(3):28.

2. Çakır İ. Determination of some probiotic properties on Lactobacilli and Bifidobacteria. Ankara University Thesis of PhD., 2003, pp.24.
3. Conter M, Muscariello T, Zanardi E, Ghidini S, Vergara A, Campanini G, *et al*. Characterization of Lactic Acid Bacteria Isolated From an Italian Dry Fermented Sausage. Annual Faa Medical and Veterinary Di Parma 2005; 25:167-174.
4. De Angelis M, Gallo G, Corbo MR, McSweeney PL, Faccia M, Giovine M, *et al*. Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. International Journal of Food Microbiology. 2003; 87:259-270.
5. De Man JC, Rogosa M, Sharpe M. Elisabeth Appl. Bact. 1960; 23:130-135.
6. Ekundayo FO. Isolation and identification of lactic acid bacteria from rhizosphere soils of three fruit trees, fish and ogi. International Journal of Current Microbiology and Applied Sciences. 2014; 3(3):991-998.
7. Engle-Stone R, Nankap M, Ndjebayi AO, Erhardt JG, Brown KH. Plasma ferritin and soluble transferrin receptor concentrations and body iron stores identify similar risk factors for iron deficiency but result in different estimates of the national prevalence of iron deficiency and iron-deficiency anemia among women and children in Cameroon. J Nutr. 2014; 143(3):369-77.
8. Famularo G, De Simone C, Pandey V, Sahu AR, Minisola G. Probiotic lactobacilli: an innovative tool to correct the malabsorption syndrome of vegetarians? Medical Hypotheses 2005; 65:1132-1135.
9. Gilliland SE, Staley TE, Bush LJ. Importance of the bile tolerance of *Lactobacillus acidophilus* used as dietary adjunct. J. Dairy Sci. 1984; 67:3045-3051.
10. Greiner R, Haller E, Konietzny U, Jany KD. Purification and characterization of phytase from *Klebsiella terrigena*. Arch. Biochem. Biophys 1997; 341:201-206.
11. Kavitha JR, Devasena T. Isolation, Characterization, Determination of Probiotic Properties of Lactic Acid Bacteria from Human Milk. Journal of Pharmacy and Biological Sciences. 2013; 7(3):1-7.
12. Lopez HW, Ouvry A, Bervas E, Guy C, Messager A, Demigne C, *et al*. Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. Journal of Agricultural and Food Chemistry. 2001; 48:2281-2285.
13. Lorca GL, Font de Valdez G. Acid tolerance mediated by membrane ATPases in *Lactobacillus acidophilus*. Biotechnol. Lett. 2001; 23:777-780.
14. Maren M, Ines ME, Isabelle A, Richard FH, Leo Meile. Phytic acid degrading lactic acid bacteria in tef-injera fermentation Int jounal of Food Microbiology, 2014.
15. Maryam M, Sabihe S, Shahram D, Mahmoud SZ, Ali A. Investigation of acid and bile tolerance of native lactobacilli isolated from fecal samples and commercial probiotics by growth and survival studies Iranian journal of biotechnology. 2009; 7(4):233.
16. Mittal A, Singh G, Goyal V, Yadav A, RaiAneja K, Kumar GS. Isolation and biochemical characterization of acido-thermophilic extracellular phytase producing bacterial strain for potential application in poultry feed. Jundishapur J Microbiol. 2012; 4(4).
17. Nabil MKE, Amany SY, Mariam GMM. Isolation, purification and characterization of phytase from *Bacillus subtilis* MJA. African Journal of Biotechnology. 2013; 12(20):2957-2967
18. Nielsen MM, Damstrup ML, Hansen A. An optimised micro-titer plate method for characterisation of endogenous rye phytase under industrial rye bread making conditions. European Food Research and Technology 2008; 227(4):1009-1015.
19. Nolla NP, Marie MKS, Marlyne J, Tetanye E, Inocent G. Assessment of nutritional status of preschool children in the Bangang rural community, Cameroon International Journal of Biotechnology and Food Science. 2004; 2(2):44-52
20. Palacios MC, Haros M, Rosell CM, Sanz Y. Characterization of an acid phosphatase from *Lactobacillus pentosus*: regulation and biochemical properties. Journal of Applied Microbiology. 2005; 98:229-237.
21. Palacios MC, Haros M, Sanz Y, Rosell MC. Selection of lactic acid bacteria with high phytate degrading activity for application in whole wheat bread making. LWT Food Science and Technology 2008; 41(1):82-92.
22. Pasquale R, Maria LVC, Mattia PA, Vittorio C, Salvatore M, Giuseppe S, *et al*. Fresh-Cut Pineapple as a New Carrier of Probiotic Lactic Acid Bacteria Biomed Res Int. 2014; 30(9):183.
23. Ponnala R, Prakash MH. Screening, selection and characterization of phytic acid degrading lactic acid bacteria from chicken intestine International Journal of Food Microbiology. 2009; 133:129-134.
24. Suk Hee Jung, Joung Whan Park, Jae Cho, Nam Keun Lee, In-Cheol Yeo, Byung Yong Kim, *et al*. Characterization of Lactic Acid Bacteria Isolated from Sauce-type Kimchi. Prev Nutr Food Sci. 2012; 17(3):217-222. doi: 10.3746/pnf.2012.17.3.217
25. Yetti M, Rina D, Neni G, Gita C. Identification Characterization and Production of Phytase from Endophytic Fungi International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering. 2010; 4(5).