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Effects of Ginger Fortification of Ogi on Lactic Acid Bacteria and Aflatoxin Levels

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Research Article

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Abstract

The influence of ginger on Lactic Acid Bacteria (LAB) load / some probiotic properties, aflatoxin levels and acceptability of ogi (maize pap) co – fermented with ginger was studied. Various quantities (0, 4, 6, 8, and 10 grams) of fresh ginger were incorporated into 400 grams fermenting maize at the beginning of fermentation. Changes in Total Titratable Acidity (TTA), pH and LAB load were determined using standard procedures. Sensory evaluation was done using nine point hedonic scales. Enzyme Linked Immunosorbent Assay (ELISA) kit was used to determine the total aflatoxin level. Isolates were screened for probiotic properties and identified using standard procedures. The pH values ranged between 4.90 and 7.20 in non ginger fermenting maize (control) and from 3.80 – 7.10 in the ginger fermenting maize. TTA ranged between 0.11 % and 0.70 % in the control and from 0.12 – 0.72 % in the ginger fermenting maize. LAB population increase significantly at $p < 0.05$ and ranged from 3.0 x 104cfu/mL – 8.2.0 x 104cfu/mL in the control and from 3.3 x 104cfu/mL - 8.8 x 104cfu/mL in the ginger samples. LAB isolates were identified as Lactobacillus fermentum, L. plantarum, L. acidophilus and L. brevis and they possessed potentials to assimilate cholesterol except L. brevis. They were also able to withstand pH values of 2, 3, 4, and 7 and tolerate 0.1, 0.5 and 1.0 % bile concentrations after 24 h. Aflatoxin levels decreases significantly from 50 ppb to 2.0 ppb in control and to 1.8 ppb in the ginger samples. High quantity of ginger reduced acceptability. This study demonstrated that ginger has no negative effects on LAB and aflatoxin level reduction potential of LAB, its use as additive in ogi could be encouraged.

Keywords: Ginger; Fermentation; Ogi; Aflatoxin; Lactic Acid Bacteria Load

Introduction

Ogi (maize pap), a fermented cereal porridge produced by lactic acid fermentation of maize (Zea mays), guinea corn (Sorghum bicolor) or millet (Pennisetumtyphodenum), is a dietary staple food for children and adults in Nigeria often flavoured with ginger, garlic and onion [1]. It is produced by soaking corn in water for 2 to 3 days, followed by milling and sieving through a screen mesh. The sieved material is allowed to

sediment and ferment, and the fermented product marketed as wet cakes locally known as ogi [2]. Different genera of microorganisms have been isolated during fermentation of maize into ogi, including various yeast and mould species, and Lactobacillus spp [3].

Lactic acid bacteria (LAB) are integral to many African fermented foods [4]. Today, LAB are a focus of intensive international research for their essential role in most fermented foods and ability to produce various antimicrobial compounds, thereby promoting probiotic properties [5].

Lactobacillus species are the predominant organisms involved in the fermentation of cereal based foods and beverages in Africa. These organisms are reported to have bacteriostatic, bactericidal, viricidal, anti-leukaemic and antitumor effects in the consumer [6]. Beneficial starter cultures are not usually used in the fermentation of traditional cereal based foods and beverages. However, it is reported that fermented foods have a probiotic potential [7] due to the probiotic Lactobacillus species that may be contained in them, some of which are of human intestinal origin [8].

Mycotoxins are natural secondary metabolites produced by fungi on agricultural commodities in field and during storage, under a wide range of climatic conditions and are of significance in food safety [9]. The food borne mycotoxins that are likely to be of great significance to human health in tropical developing countries are the fumonisins and aflatoxins [10]. Among these mycotoxins, aflatoxin has gained considerable attention because it is a more toxic and potent carcinogen even in small quantities [11] and it is also the mycotoxin of public health importance within the West African region.

Aflatoxins are toxic metabolites produced by a variety of moulds such as Aspergillus flavus and Aspergillus parasiticus. They are carcinogenic and can be present in grains, nuts, cottonseeds and other commodities associated with human food or animal feeds. Crops may be contaminated by one or more of the four following sub-types of aflatoxin: B 1, B 2, G 1 and G 2. Aflatoxin B 1 is the most toxic and frequently detected form. The other types present a significant danger at a high level concentration. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Rey's syndrome and chronic hepatitis [12].

Accurate and rapid determination of the presence of aflatoxin in commodities is of paramount importance. The Standards Organization of Nigeria (SON) sets standards on many food commodities, taking into account global standards as well as national production and target export markets. While it is generally recognized globally that there is no safe level of aflatoxin exposure, SON has set the maximum acceptable limit for maize grain at 4 ppb for total aflatoxins and 2 ppb for aflatoxin B1 [12].

Ginger (zingiber officinale roscoe, Zingiberacae) is an important medicinal plant which indigenous to several countries and is consumed worldwide as a spice and flavouring agent from the ancient time [13]. It has some tremendous beneficial effect to human body to cure various types of diseases due to its many chemical constituents such as Amaldehyde, Gingerol, Shogaol, and Paradol. Ginger is rich in antioxidants and phytochemicals with anti-inflammatory, antimicrobial and anticancer properties. It has been used as a condiment and for the treatment of ailments for many years.

However, there are limited studies on the antioxidant and scavenging power of ginger used to ferment ogi on aflatoxin levels and Lactic Acid Bacteria; one of the predominant fermenter and health benefiting organism in ogi. Since heavily aflatoxin-contaminated grain is a common feature in Nigerian markets, and ogi been food of many in Nigeria, this study therefore was aimed at determining the effect of ginger on lactic acid bacteria load in ogi, its aflatoxin reducing potential and other probiotic potentials when it is used as a co-fermenter of maize naturally infected with aflatoxin used for ogi production.

Materials and Methods

Collection of Maize and Ginger Samples / Preparation

Maize and Ginger for this study were obtained from Animal Care Consult, Ogere Remo, Abeokuta, Ogun State, Nigeria. The grains were sorted to remove dirts and spoilt ones from the healthy ones. Ginger was prepared according to modified methods of Olubamiwa AO [14]. Four hundred grams of maize were weighed into five bowls each and 1000ml of water added with ginger of 0 (no ginger), 4, 6, 8 and 10 grams incorporated respectively. Given rise to five different samples (A- 400g maize + No ginger [control], B – 400g maize + 4g ginger. C – 400g maize + 6g ginger, D – 400g maize + 8g ginger and E – 400g maize + 10g ginger). Each bowl of maize grains was fermented 24 h, 48 h, 72 h and 96 h respectively. For Aflatoxin, the zero hour samples were milled and analysed immediately and were used as control.

Acidity Changes

Changes in the pH and TTA were assessed in triplicate at 12h interval throughout the fermentation period. The pH was determined using a Metrohm 620 pH meter (Metrohm Herisau, Switzerland) with a reference glass electrode. The pH meter was calibrated prior to each reading with standard buffer 7. the TTA was analyzed by titrating 20ml of the supernatant against 0.1N NaOH until pH 8.30 was attained. The titer volume of each homogenates was multiplied by 0.09 to give the percentage TTA as lactic acid [15].

Acid Tolerance

This was carried out by a modified method described by Haller, et al. (2001) [16]. The isolated lactobacilli were subjected to primary screening for acid tolerance in MRS broth adjusted to pH 2.5 with 1N HCl for 90 min at 37°C. The determination of survival was performed by single streaking on MRS agar plates, and the growth was observed after 24 h of anaerobic incubation at 37°C. The growths of isolates on the agar were considered to be acid tolerant strains. These strains were selected and cultivated in MRS broth under anaerobic atmosphere at 37°C.

Cultures (106 cfu/mL) were inoculated in 10 mL of 0.05 M sodium phosphate buffer adjusted to pH 2.0, 3.0, 4.0 and 7.0 with 1 N HCl. Samples were incubated at 37°C for 2 h. Cultures were serially diluted using phosphate buffer pH 7.0. Appropriate dilution was plated on MRS agar for determination of viable cells after 24 h of incubation. The survival rate was calculated as the percentage of colonies grown on MRS agar compared to the initial cell concentration. Each experiment was performed in triplicate [16]. Initial counts were standardized using McFarland standard to 1.5 x 106 cfu/mL before exposing isolates to variable bile concentrations.

Bile Salt Tolerance

To determine the effect of bile salts on the growth rate of LAB a modified method described by Liong and Shah (2005) [17] was employed. The MRS broths at concentrations of 0.5 %, 1.0 % and 1.5 % (w/v) of oxgall, Difco, Detroit, USA was prepared and dispensed in 10 mL volumes and sterilized by heating at 121°C for 15 min. Pure cultures of each of the isolated LAB (106 cfu/mL) were inoculated into each medium, incubated at 37°C for 24 h., and growth monitored. The survival rate of each strain was expressed as the percentage of viable cells in the presence of bile salt compared to that without bile salt (I.e, initial count before exposure to bile and final counts after exposure). The experiment was performed in triplicate and the mean values were calculated [17].

NOTE: Before exposure to different concentrations of bile, counts from each isolates were adjusted to 1.30 x 106cfu/mL using A 0.5 McFarland standard.

Bile tolerance= (No. of viable cells with bile salts)/ (No. of viable cells without bile salts) x 100

Cholesterol-Lowering Property

The ability of isolates to assimilate cholesterol was determined by a modified method described by Dora and Glenn (2002) [18].

A 9.9 ml aliquot of MRS broth containing 0.4 % bile salt (w/v) and 0.01 % (w/v) cholesterol was inoculated separately with 0.1ml overnight culture of each of the isolates. The inoculated bottles were then incubated at 37°C under anaerobic conditions for 18 h. The bacterial cells were then removed from the culture broth by centrifugation at 4000 rpm for 20 min and the supernatant was used directly for determination of cholesterol level. Spectrophotometer was used to determine the level of turbidity of each isolates supernatant. Less turbidity implies high reduction in cholesterol while high turbidity implies low reduction.

Sensory Evaluation

Sensory evaluation was done according to modified method described by Iwe, 2002 [19] using a ten member panel on a nine point Hedonic scale ranging from 9 = high acceptability to 1 = low acceptability.

ELISA test for Total Aflatoxin Level

The total aflatoxin level in was detected and quantified using Enzyme Linked Immunosorbent Assay (ELISA) kits (Agra Quant Aflatoxin. Singapore). The kit was used according to the manufacturer's instruction.

Bacterial Genomic DNA Analysis

Bacterial genomic DNA was extracted using the protocol stated by Trindade, et al. (2007) [20].

Statistical Analysis

Data obtained were inputted using SPSS (version 17.0). Significant differences between means ($p < 0.05$) were separated using Duncan multiple range test.

Results & Discussion

A significant decrease in pH value (Figure 1) throughout the fermentation period was noticed, while there was a significant increase (Figure 2) in the total titratable acidity (TTA) in all the samples. Steady decrease in pH and increase in TTA during fermentation of maize without ginger into ogi has been reported by Omemu, (2011) [3] this might be as a result of the consumption of free sugar in the sample and production of lactic acid by fermentative organisms responsible for the fermentation of ogi. In this study, the decrease in pH and TTA (Figures 1 and 2) was higher in the ginger samples compared to the non ginger samples.

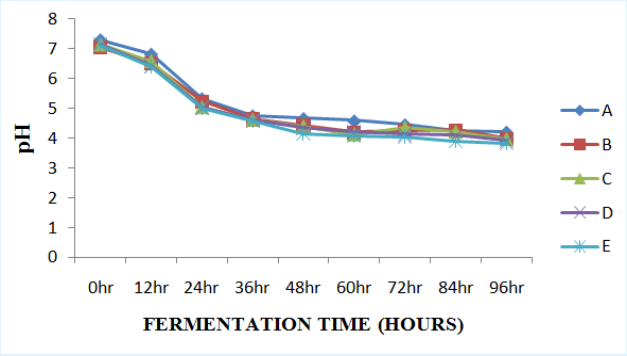

Figure 1: Effect of fermentation on pH of ginger and non – ginger fermented maize. KEY: A= Control (400 grams Maize No Ginger), B= 400 grams maize + 4 grams Ginger,,C=400 grams maize + 6 grams Ginger, D= 400 grams maize + 8 grams Ginger, E= 400 grams maize + 10 grams Ginger.

Values are means of triplicate treatments.

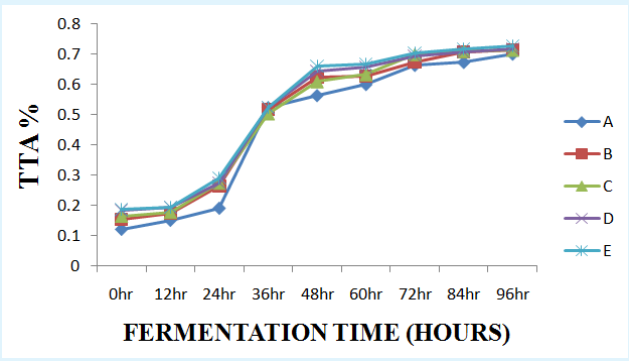

Figure 2: Effect of fermentation on pH of ginger and non – ginger fermented maize. KEY: A= Control (400 grams Maize No Ginger), B= 400 grams maize + 4 grams Ginger,,C=400 grams maize + 6 grams Ginger, D= 400 grams maize + 8 grams Ginger, E= 400 grams maize + 10 grams Ginger.

Values are means of triplicate treatments.

The antimicrobial activity of ginger has been reported [21]. But in the present study, ginger did not negatively affect LAB population as show over the 96 h fermentation period (Table 1). Increase in LAB growth during fermentation of maize without ginger has been reported [3], but in the present study, the ginger samples recorded more growth compared to the non ginger samples (Table 1), this might be as a result of the stimulating effects of ginger used on the growth of the LAB isolates resulting in the enhanced acid production [22].

Time Interval (Hours) / LAB Counts (Cfu/Ml)					
***Ogi*/Ginger Samples**	**0**	**24**	**48**	**72**	**96**
A	0	3.0 ± 0.033^{a}	5.8 ± 0.033^{b}	7.6 ± 0.033^{cd}	8.2 ± 0.067^{d}
B	0	3.3 ± 1.333^{ab}	5.9 ± 0.33^{b}	7.7 ± 0.333^{c}	8.5 ± 1.454^{d}
C	0	3.4 ± 2.000^{ab}	5.8 ± 0.66^{b}	7.7 ± 0.333^{cd}	8.8 ± 0.882^{d}
D	0	3.4 ± 1.453^{ab}	5.9 ± 0.333^{b}	7.7 ± 0.578^{c}	8.8 ± 0.882^{d}
E	0	3.5 ± 1.202^{ab}	6.0 ± 0.882^{b}	7.8 ± 0.577^{c}	8.7 ± 1.202^{d}

Table 1: Lactic Acid Bacteria Count of Fermenting Maize Containing Different Quantities of Ginger.
Values are means of triplicate treatments ± standard error.
KEY: A= control (400 grams maize no ginger), B= 400 grams maize + 4 grams ginger, C= 400 grams maize + 6 grams ginger, D= 400 grams maize + 8 grams ginger, E= 400 grams maize + 10 grams ginger.

A decrease in total aflatoxin level of naturally aflatoxin contaminated maize was also studied and a decrease in total aflatoxin level was recorded among all the samples (Table 2). Reduction in aflatoxin level in fermented naturally aflatoxin contaminated maize without ginger has been reported by Oluwafemi and Ikeowo, but in the present study, a more decrease was noticed among the ginger samples compared to the non ginger sample.

Time (hrs)	Total Aflatoxin level (ppb) at different quantities of Ginger			
	A	B	C	D
0	50	50	50	50
24	4.0±0.23	4.0±0.23	4.0±0.23	4.0±0.23
48	3.2±0.17	3.0±0.17	3.0±0.23	3.0±0.15
72	2.0±0.17	1.9±0.17	2.0±015	1.9±0.23
96	2.0±0.17	1.9±0.23	1.9±0.17	1.8±0.16

Table 2: Aflatoxin levels of fermenting maize containing different quantities of ginger.
Values are means of triplicate treatments ± standard error.

The use of ginger as additive in ogi has lots of benefits ranging from health [23] to improvement of ogi shelf life, but at high concentrations in ogi, people tends to dislike it due to its peppery taste (Figure 3) as shown in this study.

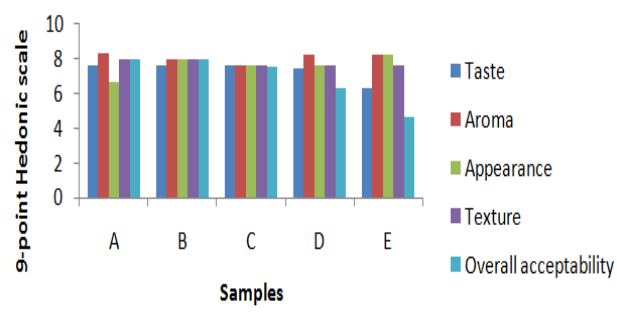

Figure 3: Effect of different concentrations of ginger on sensory quality of fermented maize (Ogi).

KEY; A= control (400 grams maize no ginger), B= 400 grams maize + 4 grams ginger, C= 400 grams maize + 6 grams ginger, D= 400 grams maize + 8 grams ginger, E= 400 grams maize + 10 grams ginger. 9= Excellent, 8= Very Good, 7= Good, 6= Poor, 5= Very Poor

Survival of bacterial strains in low pH and Bile salt is a more accurate indication of the ability of strains to survive passage through the stomach. Result (Tables 3 and 4) shows that isolates could survive pH and bile concentrations after 24 h exposure to pH of 7, 2, 3 and 4 as well as 0.1, 0.5 1% bile, although a decrease in growth was noticed among all the samples. Overall, the ginger samples recorded more growth compared to the non ginger samples.

Isolates	Bacterial count (× 10^6 CFU/mL) after 24 hrs of acid exposure																			
	pH7					pH4					pH3					pH2				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
L. plantarum	1.53±0.03^{b}	1.83±0.04^{b}	1.71±.03^{a}	1.73±0.02^{b}	1.83±0.02^{b}	1.46±0.03^{b}	1.80±0.03^{ab}	1.59±0.02^{ab}	1.61±0.02^{b}	1.75±0.03^{b}	1.24±0.02^{a}	1.74±0.03^{ab}	1.48±0.03^{bc}	1.43±0.03^{b}	1.60±0.04^{b}	1.22±0.03^{c}	1.63±0.09^{a}	1.40±0.03^{c}	1.40±0.03^{c}	1.37±0.04^{a}
L. fermentum	1.70±0.03^{c}	1.71±0.03^{a}	1.70±0.03^{c}	1.80±0.03^{b}	1.84±0.03^{b}	1.63±0.02^{bc}	1.51±0.02^{ab}	1.63±0.02^{b}	1.71±0.02^{c}	1.80±0.02^{b}	1.60±.03^{b}	1.48±0.03^{bc}	1.60±0.03^{b}	1.70±0.02^{bc}	1.75±0.01^{b}	1.35±0.03^{a}	1.40±0.03^{c}	1.35±0.03^{a}	1.60±0.02^{a}	1.65±0.03^{a}
L. acidophilus	1.73±0.03^{a}	1.40±0.03^{b}	1.62±0.03^{a}	1.67±0.04^{ab}	1.87±0.04^{c}	1.60±0.03^{a}	1.34±0.03^{c}	1.53±0.03^{b}	1.60±0.02^{a}	1.80±0.02^{c}	1.55±0.03^{a}	1.30±0.04^{b}	1.52±0.03^{a}	1.60±0.02^{a}	1.37±0.04^{b}	1.53±0.03^{a}	1.27±0.02^{a}	1.44±0.03^{b}	1.50±0.02^{b}	1.23±0.02^{a}
L. brevis	1.43±0.02^{a}	1.48±0.02^{b}	1.51±0.02^{c}	1.55±0.02^{cd}	1.67±0.02^{d}	1.37±0.04^{a}	1.42±0.02^{b}	1.43±0.02^{b}	1.45±0.03^{c}	1.50±0.03^{c}	1.31±0.01^{b}	1.31±0.02^{a}	1.33±0.02^{a}	1.35±0.04^{b}	1.37±0.04^{b}	1.23±0.05^{c}	1.20±0.02^{a}	1.22±0.02^{b}	1.22±0.02^{b}	1.25±0.02^{c}

Table 3: Effect of pH on the viability of the isolated LAB strains
Values are means of triplicate treatment ± standard error.
(F=46.823, P=0.001, superscript a, b, c, d are significantly different ($p < 0.05$).
KEY: A= Control (400 grams Maize No Ginger), B= 400 grams maize + 4 grams Ginger,,C= 400 grams maize + 6 grams Ginger, D= 400 grams maize + 8 grams Ginger, E= 400 grams maize + 10 grams Ginger

Isolates	Bacterial count (×10^6/CFUmL)after 24 hrs of exposure to different bile concentrations														
	Bile 0.1%					Bile 0.5%					Bile 1%				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
L. plantarum	1.61± 0.02^b	1.58± 0.04^b	1.53± 0.02^b	1.80± 0.02^c	1.43± 0.02^a	1.53± 0.03^{ab}	1.40± 0.03^b	1.43± 0.03^a	1.71± 0.01^b	1.40± 0.03^a	1.50± 0.03^a	1.37± 0.03^a	1.37± 0.04^a	1.51± 0.03^a	1.37± 0.03^a
L. fermentum	1.73± 0.03^b	1.51± 0.02^b	1.71± 0.03^c	1,48± 0.02^b	1.23± 0.03^b	1.43± 0.03^a	1.43± 0.02^a	1.62± 0.00^b	1.44± 0.03^b	1.11± 0.02^a	1.40± 0.03^a	1.37± 0.02^a	1.53± 0.03^a	1.43± 0.02^b	1.38± 0.02^c
L. acidophilus	1.67± 0.03^b	1.39± 0.03^c	1.67± 0.03^b	1.43± 0.02^b	1.23± 0.03^a	1.55± 0.03^a	1.31± 0.03^b	1.59± 0.03^a	1.40± 0.03^b	1.27± 0.04^{ab}	1.51± 0.02^a	1.39± 0.03^c	1.56± 0.02^a	1.32± 0.02^a	1.35± 0.03^b
L. brevis	1.37± 0.02^c	1.37± 0.02^c	1.34± 0.02^a	1.25± 0.02^a	1.25± 0.02^a	1.32± 0.02^b	1.32± 0.02^b	1.41± 0.03^c	1.41± 0.03^c	1.41± 0.03^c	1.12± 0.02^{ab}	1.34± 0.03^b	1.34± 0.03^b	1.34± 0.03^b	1.30± 0.03^a

Table 4: Bile Tolerance of LAB (Cfu/mL) at Different Bile Concentrations.
Values are means of triplicate treatment ± standard error.
F=35.823, P=0.001, superscript a, b, c, d are significantly different (P < 0.05).
KEY: A= Control (400 grams Maize No Ginger), B= 400 grams maize + 4 grams Ginger,,C= 400 grams maize + 6 grams Ginger, D= 400 grams maize + 8 grams Ginger, E= 400 grams maize + 10 grams Ginger.

This might be as a result of the stimulating effects of ginger used on the growth of the LAB isolates resulting in the enhanced acid production [22].

These tolerances are an indication that the isolates could successfully transit the human stomach and may be capable of reaching the intestinal environment to carry out their functioning effects.

Another phenomenon related to the presence of the deconjugation activity is the reduction of serum cholesterol. The removal of cholesterol by lactobacilli in vitro could be due to an uptake or assimilation of cholesterol by bacterial strains. Liong MT, et al. demonstrated that a portion of the cholesterol assimilated by Lactobacillus strains was incorporated into the cellular membrane [17]. Hyeong-Jun L, et al. Reported that strains of lactobacilli tested removed 31.5 to 58.5% cholesterol in the growth medium. In this study, L. plantarum shows the highest level (41.23%) of cholesterol reduction (Tables 5 and 6) but the percentage of cholesterol removal varied considerably among strains. L. brevis showed no reduction or assimilation of cholesterol at the course of this study [24, 25].

Isolates	Percentage (%) absorbance				
	A	B	C	D	E
L.plantarum	15.25	18.93	14.69	41.23	12.71
L. fermentum	11.3	11.3	13.28	20.9	13.28
L. acidophilus	17.51	12.15	17.51	20.9	20.9
L. brevis	-	-	-	-	-

Table 5: Cholesterol Assimilation of Isolated LAB on Fermented Maize.
KEY - = REDUCTION += NO REDUCTION.A= Control (400 grams Maize No Ginger), B= 400 grams maize + 4 grams Ginger, C= 400 grams maize + 6 grams Ginger, D= 400 grams maize + 8 grams Ginger, E= 400 grams maize + 10 grams Ginger.

Isolate code	Description of closest identity	E- Value	% Identity	Accession number of similarity sequences
1	*Lactobacillus plantarum*MF1298	0	99%	CP013149.1
2	*Lactobacillus fermentum*	0	99%	FJ462686.1
3	*Lactobacillus acidophilus* EMBS082	0	99%	KC150145.1

Table 6: The similarity of bacterial sequences with sequences obtained from NCBI database gene bank.

Conclusion

Ginger does not have adverse effect on Lactobacilli isolated from ogi fermented with ginger. Hence, the use of ginger in ogi would decrease the chances of food poisoning, reduce the risks of food contamination, protect consumers from aflatoxin contamination and improve health status.

The use of ginger as food additive should be encouraged since it does not have a negative effect on LAB growth.

It was observed that, the Lactobacillus strains associated with ogi fermented with ginger also contain potential probiotic strains with cholesterol-lowering properties.

Therefore, the use of ginger in ogi and probably other food items would decrease the chances of food poisoning, reduce the risk of food contamination, protect the consumer from different food-borne diseases and improve health status by using a small quantity of it. It is therefore concluded that ginger do not reduce but enhance the growth LAB and subsequently lead to aflatoxin level reduction.

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