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Assessment of probiotic potentials of deger sold in tamale

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ABSTRACT

"Deger", a fermented milk product is gaining popularity among consumers in Tamale Metropolis and other cities in Ghana. Among the many advantages of fermented foods, the probiotic activity of the fermenting microorganisms cannot be overemphasized. This work assessed the probiotic potential of deger using lactic acid bacteria (LAB), major probiotic organisms as the indicator. The viable LAB counts of samples purchased from nine different sources in Tamale were enumerated by serial dilution and pour-plating in selective media, MRS-S. There was significant difference ($p<0.05$) in mean LAB count of the deger samples from different sources with sources B, E and F recording the highest mean LAB counts of $2.86\pm0.03\times10^8$, $2.53\pm0.11\times10^8$ and $2.95\pm0.04\times10^8$ cfu/ml respectively whilst source G recorded the least significant counts of $1.15\pm0.05\times10^8$ cfu/ml . Because there is spontaneous fermentation in deger production, twenty isolates were randomly selected from the samples and identified by standard morphological, physiological and biochemical methods. The isolates were identified as Lactobacillus delbrueckii (30%), Lactobacillus casei (25%), Lactobacillus leichmannii (25%) Lactobacillus acidophilus (15%) and Lactobacillus fermentum (5%). It is concluded that deger consume in Tamale is a promising probiotic food. This is in view of fact that the minimum serving size of 150 ml from the source with the least mean LAB counts of $1.15\pm0.05\times10^8$ cfu/ml contained $1.731.15\times10^{10}$ viable cells, and that some strains of LAB isolated from deger namely L. delbrueckii, L. acidophilus L casei and L. fermentum have documented probiotic activities.

Keywords: Probiotics, Lactic acid bacteria (LAB), "Deger"

INTRODUCTION

Fermented foods and beverages constitute about 20-40% of world's food [9,16]. Apart from their numerous advantages such as higher nutritional content, less anti-nutritive substance, prolonged shelf-life of the food, and reduced risk of food borne diseases, fermented food and beverages can have beneficial health effect when the fermenting microorganisms possess probiotic activities [14,16,21]. Probiotics are live microorganisms which, when administered in adequate amounts confer a health benefit to the host [9]. Some documented effects of probiotics are prevention of intestinal infections, alleviation of lactose intolerance, strengthening of innate immune system, lowering of serum cholesterol, reduction in the risk factors for colon cancer, enhancement of bowel motility, control of irritable bowel syndrome and control of inflammatory bowel diseases [9,22,25]

Strains of *Lactobacilli* or lactic acid bacteria (LAB), *Bifidobacteria*, *Enterococci* and yeast are commercially used as probiotics but *Lactobacilli* remain the most commonly used microorganisms [15,16,20,28]. Coincidentally, the use of lactic acid bacteria in the production of fermented foods dates back to several thousands of years [21].

The quantity of probiotic organism required to effect its activity depends on the strain and dosage but recommended dosage for probiotics remains equivocal. However the suggested dosages seem to fall within the range of 10^9 to 10^{10} viable cells for bacteria strains [8,15,26].

"Deger", "Burkina" or "nunu de fura" is a fermented milk drink which is gaining popularity in most cities in Ghana including Tamale Metropolis in Northern Region [1]. It has fermented cow milk and millet flour or "fura" as the major constituents. According to the producers, the freshly drawn unpasteurized cow milk is sieved and allowed to undergo spontaneous fermentation overnight to 24 hours at room temperature before millet flour or fura and other ingredients are added. A slightly variant method in which the milk is inoculated with a little of leftover fermented milk has also been reported by [6]. It is usually tied in rubber bags or packaged in plastic containers. It is consumed immediately or stored in refrigerator for maximum period of fortnight [1]. This work aimed at enumerating the lactic acid bacteria populations as an indicator of probiotic counts of deger sampled from different sources and identify the predominant LAB species present to assess their potential probiotic activities.

MATERIALS AND METHODS

Sample collection

Deger samples were purchased from nine (9) different sources (seller/producers) in Tamale Metropolis and labeled A to I. Three (3) samples measuring about 250 ml each were obtained from each source and kept under ice and transported to Spanish Laboratory of University for Development Studies, Nyankpala Campus for analysis.

Fig. 1. A deger sample measuring about 250 ml

Media preparation

De Man, Rogosa Sharpe sorbic acid- agar media (MRS-S) was prepared as described by [5,7] with slight modification. An amount of 1.848 g/L of potassium sorbate was added to a liter of MRS agar and autoclaved for 15minutes at 121 $^{\circ}$C.

Enumeration of LAB counts

Serial dilution and pour plate techniques were employed to enumerate LAB counts of the samples under a laminar flow hood (Envair BB4 4HX). Each dilution was plated in replicate. Samples from the same source were pooled together in a 1 liter Erlenmeyer flask and 10 ml was pipette and suspended in 90 ml of phosphate buffered saline (PBS) buffer in 250 ml beaker. The emulsion was stirred vigorously to mix thoroughly. One milliliter (1) of the suspension was serially diluted in test tubes (10^{-2} – 10^{-9}), each containing 9 ml of PBS buffer. A milliliter of each dilution was transferred into corresponding Petri dishes and about 15 ml of the molten media, cooled to 45 °C was added to it and swirled to mix well in a laminar flow hood. It was then allowed to solidify and the plates incubated inverted in 5 % CO_2 at 37 °C in carbon dioxide incubator for 48 hrs. Viable colonies on each plate within the range of 30 to300 colonies (statistically viable), were counted under magnified colony counter and data recorded as colony forming units per ml (cfu/ml). The number of colony forming units per ml of the sample was calculated as follows;

CFU/ ml= CFU x dilution factor x 1/aliquot.

GenStat 4TH edition was used to compute the significant difference and L.s.d between the mean (CFU/ ml) of the samples at a significant level of 5 %. Microsoft excel was used to plot graphs.

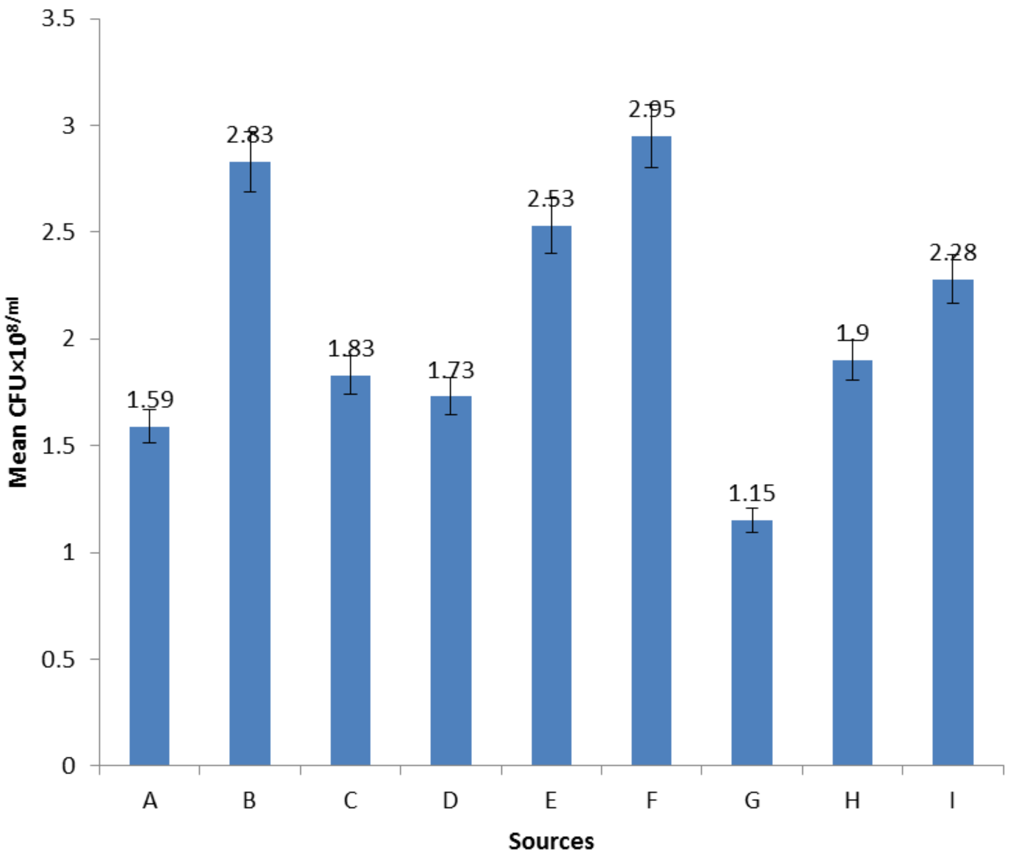
Isolation and Identification of LAB isolates
Twenty (20) colonies were randomly selected from the sample plates and streaked on fresh MRS-S agar plates and incubated in CO_2 incubator at 37 °C. The cultures were sub-cultured to obtain pure cultures. Pure cultures were maintained in a sterilised MRS-S broth and slants in test tubes and kept at -20 °C.

Morphological and biochemical characterization were carried out and the isolates identified with the aid of Bergey's manual of determinative bacteriology [2,4]. Colony morphological characteristics such as size, pigmentation, form, surface, opacity, texture, elevation, and margin were recorded.

Cell morphological, physiological and biochemical test carried out were Gram staining, spore formation, acid fast, catalase, sulphide-indole-motility, carbohydrate fermentations (glucose, mannitol, sucrose, lactose, fructose, galactose, maltose and starch), oxygen requirement and temperature tolerance tests.

RESULTS AND DISCUSSION

Fig.2. Mean probiotic counts of samples from different sources



LABs are major group of probiotics bacteria that provide health benefits to the host. They confer health benefits in a number ways such as alleviation of lactose intolerance, prevention and cure of viral, bacterial and antibiotic or radiotherapy induced diarrhoeas [12], immunomodulation, and conferring of antimutagenic and anticarcinogenic effects; and even blood cholesterol reduction [18]. LABs play an important role in the fermentation process by rapid acidification of raw materials through the production of organic acids and therefore found in fermented foods including milk products [17]. The result of this study indicates the presence of LAB in deger. This is in accordance with the work by [3] and [19], that LAB are predominant in fermented milk products.

Deger production in Tamale is a small-scale food processing industry and the producers tend to be the sellers. Deger samples were taken from nine (9) different sources. There were significant differences ($p<0.05$) in mean probiotic counts of deger samples from different sources. From Fig.1, sources B, E and F recorded the highest mean counts of

2.86±0.03×10^8, 2.53±0.11×10^8 and 2.95±0.04×10^8 cfu/ml respectively with no significant difference between them (L.S.D = 2.61x10^7). Sources A, C, D, H and I produced the second highest mean probiotic counts of 1.59±0.13×10^8, 1.83±0.17×10^8, 1.73±0.01×10^8, 1.9±0.08×10^8 and 2.26±0.22×10^8 cfu/ml respectively with no significant difference between them but were significantly different from B, E and F. Sample G contained the least mean counts of 1.15±0.05×10^8 cfu/ml, which was significantly different from the rest. According to [24], LAB counts of 8.7±1.8×10^8 cfu/ml was recorded at 24 hours of fermentation in the production of a very similar fermented milk product, “nunu”. The differences in probiotic counts of samples from different sources might be due to the composition and viability of the inocula for the spontaneous fermentation of the milk. Duration of fermentation and subtle differences in the production process might also be contributory factors.

Table 1. Colony Morphological characteristics of isolates on MRS-S agar plate

STRAINS	Size (mm)	colour	Nature of colony colour	Form	Surface	Opacity	Texture	Elevation	Margin
FA1	3	Cloudy	Not diffusible	Irregular	Smooth	transluscent	Viscous	Raised	Entire
FA2	2	Cloudy	Not diffusible	Circular	Smooth	Translucent	viscous	Raised	Entire
FA3	2	Whitish	Not diffusible	Circular	Smooth	Transparent	Moist	Raised	Entire
FA4	2	Cloudy	Not diffusible	Circular	Smooth	Translucent	moist	Flat	Undulate
FA5	3	Cloudy	Not diffusible	Circular	Smooth	Translucent	Viscous	Flat	Entire
FA6	1	Whitish	Not diffusible	Circular	Smooth	Transparent	Moist	Flat	Entire
FA7	2	Cloudy	Not diffusible	Circular	Smooth	Translucent	Moist	Raised	Undulate
FA8	1.5	Cloudy	Not diffusible	Circular	Smooth	Translucent	Viscous	flat	Entire
FA9	2	Whitish	Not diffusible	Circular	Smooth	Transparent	Viscous	flat	Entire
FA10	1	Whitish	Not diffusible	Irregular	Smooth	Transparent	mucoid	Raised	Curled
FA 11	3	Whitish	Not diffusible	Irregular	Smooth	Transparent	viscous	Raised	Undulate
FA12	1	Whitish	Not diffusible	Irregular	Smooth	Transparent	Viscous	flat	Undulate
FA13	1	Cloudy	Not diffusible	Circular	Smooth	Opaque	Mucoid	fat	Entire
FA14	2	Cloudy	Not diffusible	Circular	Smooth	Translucent	viscous	Raised	Undulate
FA15	3	Whitish	Diffusible	Circular	Smooth	Transparent	viscous	Raised	Entire
FA16	1	Cloudy	Not diffusible	Circular	Mucoid	Opaque	Viscous	flat	Entire
FA17	2	Cloudy	Not diffusible	Circular	Mucoid	Translucent	viscous	Flat	Curled
FA18	1	Cloudy	Not diffusible	Irregular	Mucoid	Translucent	Butyrous	Raised	Entire
FA19	1	Whitish	Not diffusible	Circular	Smooth	Transparent	Viscous	Raised	Entire
FA20	2	Cloudy	Not diffusible	Irregular	Smooth	Translucent	Butyrous	Raised	Entire

Table 2. Carbohydrate Fermentation Tests for the Isolates

Isolates	Glucose	Mannitol	Sucrose	Lactose	Fructose	Galactose	Maltose	Starch
FA1	+	_	+	+	+	+	+	-
FA2	+	+	+	-	+	+	+	-
FA3	+	+	_	+	+	+	+	-
FA4	+	+	+	-	+	+	+	-
FA5	+	+	_	+	+	+	+	-
FA6	+	_	+	_	+	+	+	-
FA7	+	+	+	-	+	+	+	-
FA8	+	_	+	_	+	+	+	-
FA9	+	_	+	_	+	+	+	-
FA10	+	+	+	-	+	+	+	-
FA11	+	+	_	+	+	+	+	-
FA12	+	_	+	_	+	+	+	-
FA13	+	_	+	_	+	+	+	-
FA14	+	+	+	-	+	+	+	-
FA15	+	+	_	+	+	+	+	-
FA16	+	_	+	_	+	+	+	-
FA17	_	+*	+	+	+	+*	+	-
FA18	+	+	_	_	+	+	+	-
FA19	+	+	_	+	+	+	+	-
FA20	+	+	_	+	+	+	+	-

Acid positive reaction (+), negative reaction (-), acid and gas positive (+)*

Twenty (20) colonies which appear distinct were randomly selected from MRS-S agar plates of the samples and streaked on fresh plates to obtain pure cultures. Morphological, physiological and biochemical test conducted revealed that all the twenty isolates were Gram-positive rods, acid-fast negative, catalase negative, non-sporulating, non-motile, indole negative, microaerophilic with fermentative metabolism. Thus they were all identified to belong to *Lactobacillus spp* in accordance with Bergey’s manual. Results for detailed morphological, physiological and biochemical tests are recorded in Tables 1, 2 and 3. Base on these results, 6 isolates (FA6, FA8, FA9, FA12, FA13 and FA16) were identified as *Lactobacillus delbrueckii* (30%), 5 (FA3, FA5, FA11, FA15 and FA19) as *Lactobacillus casei* (25%), 5 (FA2, FA4, FA10 and FA14) as *Lactobacillus leichmannii* (25%) 3 (FA1, FA18 and FA20) as *Lactobacillus acidophilus* (15%) and 1 (FA17) as *Lactobacillus fermentum* (5%). It can be suggested that these isolates are the predominant species in deger sold in Tamale. According to [6], *Lactobacillus acidophilus* and *Lactobacillus delbrueckii subs. bulgaricus* are among the predominant species involved in production of nunu. [24] reported that *Lactobacillus fermentum* was the predominant LAB isolated from nunu samples from three town in Upper East Region of Ghana. Thus the result of this work is confirmed by previous ones.

Table 3. Cell morphological and biochemical tests

Isolates	OR	CS	GS	SF	AF	CT	MT	H_2S	I	TT		
										15°C	30°C	45°C
FA1	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA2	MA	rod	+	-	-	-	-	-	-	-	+	+
FA3	MA	Rod	+	-	-	-	-	-	-	+	+	+
FA4	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA5	MA	Rod	+	-	-	-	-	-	-	+	+	+
FA6	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA7	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA8	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA9	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA10	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA11	MA	Rod	+	-	-	-	-	-	-	+	+	+
FA12	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA13	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA14	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA15	MA	Rod	+	-	-	-	-	-	-	+	+	+
FA16	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA17	MA	Rod	+	-	-	-	-	+	-	+	+	+
FA18	MA	Rod	+	-	-	-	-	-	-	-	+	+
FA19	MA	Rod	+	-	-	-	-	-	-	+	+	+
FA20	MA	Rod	+	-	-	-	-	-	-	-	+	+

positive reaction or present(+); negative reaction or absent(-); Oxygen requirement (OR); cell shape(CS) microaerophilic (MA);Gram's staining (GS); spore formation (SF); acid fast test (AF); catalase test (CT); motility test (MT); hydrogen sulphide test (H_2S); indole (I) and temperature tolerance (TT).

The quantity of probiotic organism required to effect its activity depends on the strain and dosage but recommended dosage for probiotics remains equivocal. However the suggested dosages seem to fall within the range of 10^9 to 10^{10} viable cells for bacteria strains [8,15,26]. Considering the lowest mean probiotic counts of 1.0×10^8 cfu/ml for source G, consuming the least serving size of 150 ml by volume would provide the consumer 1.73×10^{10} cfu of live LABs which is laudable.

The stomach has a pH of 1.5 to 3 and the upper intestine contains 3-5 g/L of bile salt. *L. fermentum* strains have been found to survive in these conditions supporting the idea that it act as probiotics. They are also known to be best in inhibiting the growth of pathogenic species such as *Streptococcus* and *Staphylococcus* as well as ability to reduce cholesterol levels [10,27].

L. delbrueckii produce only acid during fermentation and therefore are homofermentive bacteria. *L. delbrueckii* synthesis lactic acid in the intestinal tract which inhibits pathogenic bacteria from colonizing. *Lactobacillus casei*, also homofermentive bacteria. Some strains of *L. casei* offer protection in the intestine of people with Crohn's disease by inhibiting bacteria *E. coli* from invading and adhering to intestinal cells. *L. casei* has the ability to grow and establish colonies in the digestive tract. This reduces the duration and severity of diarrhoea in infants, as well as diarrhoea associated with antibiotics [11,29]. Some strains are also found to lessen severity of chronic constipation [21].

L. acidophilus, produce acid only during carbohydrate fermentation and therefore classified as homofermentative bacteria. The bacteria have been found in dairy products, especially milk and plant products. The ability of strains of *L. acidophilus* to help prevent pathogenic bacteria from proliferating is well documented [23]. The bacteria also prevent diarrhoea induced by antibiotics as well as lessen the severity of Chron's disease and stimulate anticarcinogenic activity reported by [13].

CONCLUSION

Deger, a popular fermented milk product consumed in Tamale Metropolis was analyzed in this work to ascertain it probiotic potential using lactic acid bacteria as an indicator. The results from LAB enumeration revealed that, generally irrespective of the source, the products contain appreciable probiotic population though it is significantly higher in deger from particular sources namely B, E and F which recorded $2.86\pm0.03\times10^8$, $2.53\pm0.11\times10^8$ and $2.95\pm0.04\times10^8$ cfu/ml respectively. Random isolation and identification showed *L. fermentum, L. delbrueckii ssp.*

bulgaricus, *L. Casei*, *L. acidophilus* and *L. leichmannii* as predominant LAB in the deger and strains of these have documented probiotic activities. Based on these findings, there can be concluded that deger is a promising probiotic food.

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