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Investigation of the Probiotic Characteristics of *Lactobacillus fermentum* isolates from Ghanaian Fermented Maize Dough

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Abstract

Isolates of dominant *Lactobacillus fermentum* from fermented maize dough were investigated for their probiotic characteristics with the purpose of selecting appropriate starter cultures to improve and enhance the quality and safety of maize dough. 21 isolates were tested for their tolerance to pH 2.5, their sensitivity to the toxic effect of bile salts, their antimicrobial activity and adhesion to Caco-2 cell lines. In general, all isolates showed good survival rates in MRS pH 2.5 and in the presence of bile salts (0.3% oxgall). The strains did not show significant growth at pH 2.5 but maintained a high cell viability. High viability and weak growth was observed for bile salts. Antimicrobial activity varied among the strains. 90% of the strains inhibited the growth of *Shigella flexneri*, 47% inhibited *Salmonella typhimurium*, 42% *Escherichia coli*, 19% *Bacillus cereus* and 19% *Listeria monocytogenes* under conditions of low organic acid production indicating the presences of other antimicrobial substances other then acid. For their adhesion potential 34% of the strains had the property to adhere to Caco-2 cells. They were not strongly adhesive compared to positive adhesive strains included as reference. For two strains verification of probiotic characteristics was carried out in a pig feeding trials. The results attained showed that one strain did not survive the maize fermentation process where as the other was recovered as the dominant *Lactobacillus* sp in the intestinal tract of the pigs. This indicated the importance of acid tolerance for probiotic cultures. The overall results indicate a rich source of strain variability in the microbiota of maize dough in which strains with probiotic characteristics can be selected.

1. Introduction

In Ghana, fermented maize dough is used to prepare a variety of maize products such as "kenkey", "banku" which are stiff gruels, and "koko" a drinkable porridge normally used as weaning food for infant feeding (Dovlo 1970). Several authors report a stable microbiota in fermented maize dough processing comprising of lactic acid bacteria and yeasts ((Halm et al., 1993; Jespersen et al., 1994), and attributes the stability to a complex of interactions involving pH, acid production and several specific antimicrobial substances (Mensah et al., 1991; Olsen

et al., 1995). *Lactobacillus fermentum* is responsible for the fermentation and various strains of this bacteria are reported to be involved (Hayford and Jakobsen, 1998). The successful use of *Lactobacillus fermentum* as a starter culture for production of "kenkey" had been reported from previous studies (Halm et al., 1996) . The antimicrobial nature of fermented maize dough has gained attention with reports on the ability of the dough to inhibit the proliferation of certain diarrhoea causing pathogens (Mensah et al., 1988, 1991). An attribute which is very important for developing countries, since diarrhoea is recognized as a major cause of morbidity and mortality among children in many developing countries including Ghana (Yartey et al., 1993). There is evidence that water after cooking of maize dough to "kenkey" can be used or is suitable for rehydration, and stops diarrhoea (Yartey et al., 1993). Products prepared from the maize dough as well as the raw dough has been confirmed to inhibit the growth of enteric pathogens (Nout et al., 1989; Mensah et al., 1991; Olsen et al, 1995; Annan-Prah and Agyeman, 1997). Several workers now report on the ability of *Lactobacillus* isolates from some selected African fermented products including "kenkey" as bacteriocin producing (Olukoya et al 1993; Olasupo et al., 1994; 1995) this accounting for some of the inhibitory properties of maize dough.

As reviewed by Holzapel et al. (1998) the role of lactic acid bacteria in human health is of considerable interest and significance. The most frequently mentioned beneficial effects of the so-called probiotic cultures as summarized by Salminen et al. (1996) include aspects such as immune modulation, gut microflora modification, adherence to the intestinal mucosa with capacity to prevent pathogen adherence, modification of dietary proteins, modification of bacterial enzyme capacity especially of those suggested to be related to tumour induction and influence on gut mucosal permeability. For the cultures to show such probiotic activities they should be resistant to the low pH of the stomach and the toxic effects of the bile salts, and they should adhere to the intestinal mucosa (Bianchi-Salvadori 1998; Holzapel et al., 1998).

We investigated the probiotic potential of *Lactobacillus fermentum* strains from maize dough for the purpose of selection of starter cultures and possibly for the development of probiotics for oral administration in diarrhoea diseases in combination with oral rehydration therapy. This work for 21 dominant *Lactobacillus fermentum* strains from different stages of maize dough fermentation, describes *in vitro* model studies taking into account of the tolerance to low ph 2.5, the toxic effects of bile acids and , their antimicrobial activity and adhesion to epithelial cells using Caco-2

cell lines as an intestinal cell model. For two strains a verification study was carried out by a pig feeding trials.

2. Materials and methods

2.1 Microorganisms, source and maintenance

The lactic acid bacteria used in this study were isolated from samples of spontaneously fermented maize dough collected from a major commercial production site in Accra, Ghana. All sampling, isolations and identification were carried out as described by Halm et al. (1993). The origin and sources of all the bacteria used in the present study are shown in Table 1. *Pediococcus acidilactici* (pediocin PA1 producer) strain was included in the study as a positive control for the antimicrobial activity. *Lb. plantarum* strain no. 299 and *Salmonella typhimurium* (TM 3931) were included as control for the cell adhesion experiments. All lactic acid bacteria were grown on de Man, Rogosa and Sharpe (MRS; Merck, Darmstadt, Germany) medium anaerobically (Anaerocult A, Merck) incubated at 30°C. For long term storage isolates were kept in MRS broth with 50% glycerol at -40°C.

All pathogenic bacteria or indicator organisms for antimicrobial activity experiments, *Shigella flexneri, Escherichia coli, Bacillus cereus, Salmonella typhimurium* and *Listeria monocytogenes* were grown on Brain heart infusion broth (BHI; Merck, Darmstadt, Germany) at 37°C aerobically and long term storage of these bacteria were in BHI with 50% glycerol at -40°C.

2.2 Tolerance to bile acid

For the 21 isolates of *Lactobacillus fermentum* examined, 1ml of overnight cultures after two previous successive transfers in 10 ml MRS broth at 37°C were inoculated into 10 ml MRS broth containing 0.3% oxgall (Difco 0128-15-0, Difco Laboratories, Detroit MI, USA). 0.3% oxgall concentration was used as it was suggested by Gilliland et al. (1984) to be the critical concentration high enough to screen for resistant strains. The inoculated MRS with 0.3% oxgall starting with an OD of between 0.07-0.09 and inoculated normal MRS broth serving as a control

Table 1 List of strains investigated

Strains	Origin/Source
Lactobacillus fermentum[c]	
Group 1	
B13	Maize Milling Machine
H11	Fermented maize (48 h)
D17	Fermented maize (72 h)
G12	Fermented maize (24 h)
G10	Fermented maize (24 h)
A7-11[a]	Fermented maize (24h)
Group 2	
A31	Steep Maize
A7	Steep Maize
C2	Steep Water (0 h)
G20	Fermented maize (24 h)
I22	Fresh Dough
A4-25[a]	Fermented maize (24 h)
Group 3	
H17	Fermented maize (48 h)
A28	Steep water
H5	Fermented maize (48 h)
A13	Steep maize
F5	Steeping Tank
F7	Steeping Tank
G6	Fermented maize (24 h)
G11	Fermented maize (24 h)
C14	Steep water (0 h)
Pediococcus acidilactici (PA2)	Christian Hansen Lab. Hørsholm, Denmark
Lactobacillus plantarum[b] 299	Pro Viva (probiotic product:Sweden)
Salmonella typhimurium TM 3931	Depart. of Vet. Microbiology, KVL, Denmark
Salmonella typhimurium	Depart. of Vet. Microbiology, KVL, Denmark

Table 1 (continued)

Strains	Origin/Source
Shigella flexneri	Depart. of Vet. Microbiology, KVL, Denmark
Bacillus cereus	Depart. of Vet. Microbiology, KVL, Denmark
Escherichia coli	Depart. of Vet. Microbiology, KVL, Denmark
Listeria monocytogenens	Depart. of Vet. Microbiology, KVL, Denmark

[a] Previously identified (Halm et al., 1993)

[b] From Johansson et al. (1993)

[c] Previously isolated and identified (Hayford et al., 1998)

were distributed into microtitre plates, four wells per sample together with a blank and incubated at 37°C. Growth was monitored at 1 h intervals for 7 h with Multiskan DWS (Lab System, Helsinki, Finland) by measuring the absorbenc at 620 nm. Viable plate count by serial dilutions technique was performed at initial (0 h) and final (7 h) in MRS and incubated at 37°C for 48 h, the examination was carried out twice at different occasions..

2.3 Tolerance to pH 2.5

Same method as above was used except organism were inoculated into MRS broth adjusted with 10N HCL to pH 2.5.

2.4 Examination of antimicrobial activity

The inhibitory effect of the 21 isolates of *Lactobacillus fermentum* from fermented maize dough against *Bacillus cereus, Listeria monocytogenes, Shigella flexneri, Salmonella typhimirium* and *Escherichia coli* were investigated using the spot and well assay technique described by Schillinger and Lucke (1989). *Lactobacillus fermentum* cultures were grown overnight in 10 ml MRS broth at 37°C. 2.5µl of culture was spotted on the modified MRS agar containing 0.2% glucose and incubated anaerobically at 30°C for 24 h. The indicator organisms (pathogens) were grown in BHI at 37°C for 24 h a 100 µl of this overnight culture was transferred into 7 ml molten BHI agar (0.7%) at 45°C. The plate where then overlayed with the

mixture of indicator organisms and molten BHI. The plates were incubated at 37°C aerobically for 24 h - 48 h. Duplicates runs were conducted, and on each plate were one positive control which was the bacteriocin producing *Pediococcus acidilactici* and a spot of media (MRS) as negative control. The well assay technique as described by Schillinger and Lucke (1989) was used for examination of culture supernatant after incubations *Lactobacillus fermentum* strains as described above. Cell free supernatant fluid (5000 x g for 15 min) was sterilized by filtration (pore size 0.2 μm, Millipore, Bradford, USA) and concentrated by lyophilization (Heto Holten lyophilizer, model FD3, Allerød, Denmark).The lyophilized precipitate was diluted in PBS and used in the concentration (× 10) of the culture supernatant. pH adjustment was made by using 1N NOAH. All the experiments were repeated twice on different occasions.

2.5 Adhesion of Lb. fermentum to Caco-2 Cell Lines

2.5.1 Maintenance and Culturing of Caco-2 cell Lines

Caco-2 cells originally isolated from a human colon adenocarcinoma were obtained from the American Type Culture Collection (Rockville, Maryland, USA). For long term storage cells were preserved in Eagle`s minimum essential medium (MEM, GibcoBRL, Life Technologies, Roskilde, Denmark) with 50% glycerol in liquid nitrogen at -196°C. Caco-2 cells were routinely cultured in 80 $cm^2$ SI culture bottles (Nunclon™ Nunc, Roskilde, Denmark) in Eagle`s minimum essential medium (MEM. GibcoBRL, Life Technologies, Roskilde, Denmark) supplemented with 20% Fetal bovine serum (GibcoBRL CAT. 10106-151, Life Technologies) which had been heat-inactivated (30 minutes at 56°C), 0.1mM non-essential amino acid (GibcoBRL, cat. 11140-035) and 0.5ml of (50mg/ml) gentamicin (GibcoBRL, cat. 15750-037) and incubated at 37°C in a water jacketed incubator with 10% carbon dioxide.

2.5.2 Preparation of Caco-2 cells for adhesion assay

For the adhesion assay, monolayer of Caco-2 cells were prepared from post confluent monolayer cells previously grown in 80cm^2 SI culture bottles. Cells were first washed twice with Dulbecco`s phospate buffered saline (D-PBS 1×) (GibcoBRL, cat. 14080-048), then trypsinized

with 3ml trypsin solution (0.5% Trypsin/EDTA solution, GibcoBRL, cat. 35400-027) for 5-10 minutes at 37°C. After 10 min cells were checked under the microscope (×200) (Inverse microscope, Will, Wiloverts, Hund, Wetzlar, Germany) for complete trypsinization (ie no adhesion of cells to the bottom of culture bottle). 5 ml of cell culture medium (MEM) were added to the trypsinized cells to stop the enzyme reaction. The concentration and viability of cells were determined by staining 100µl of cells with 100µl of Trypan Blue Stain (Sigma Cell Culture Reagent cat. T-8154, Sigma, Chemical Co., St. Louis Mo. USA) and microscopy (×200). Blue stained cell were considered non-viable, while viable cells were colourless. Concentration of cells was determined by counting a drop of stained cell solution using a haemocytometer (Neubauer improved, Germany). 3 ml of 1.5 x 10^5 cells/ml were distributed into each 35mm TC dish (Nunclon DSI) and incubated for 7 days with a change of media every 48 h till a complete monolayer was obtained.

2.5.3 Preparation of bacteria for adhesion experiment

Isolates of *Lactobacillus fermentum* and *Lactobacillus plantarum* strain 299, were subcultured twice in MRS, and *Salmonella typhimurium* (TM 3930 and TM 3931) twice in BHI at 37°C for 24 h prior to adhesion experiments. The bacterial culture was appropriately diluted with cell culture medium (MEM) to a concentration of approximately 10^8-10^9cell/ml estimated by microscopy. 120µl of this suspension was used to inoculate the Caco-2 cell culture prepared for the adhesion assay.

2.6 Adhesion Assay

Caco-2 cells in 35 mm TC dish were washed twice with D-PBS, and 3 ml MEM (without gentamicin) was put into each dish and incubated for 30 min before inoculation of bacteria. 120µl of bacteria suspension (10^8-10^9cfu/ml) prepared as previously described was used to inoculate each dish. The plates were incubated for 1 h at 37°C. After incubation, all the dishes were washed 4 times with D-PBS to release unbound bacteria. The cells were then fixed with methanol (Bie and Berntsen no. BBB22060 Rodovre, Denmark) by transferring 3 ml methanol to each TC dish (Caco-2 cells plus adhered bacteria) and incubating for 5-10 min at room temperature, followed

by removing all the methanol and addition of 3 ml of Giemsa stain solution (1:20) (Merck.no.9204, Darmstadt, Germany). After 30 min the Giemsa strain was removed by several rinses with distilled water till no colour was observed in the washing solution. TC dishes were dried in an incubator (Termaks 6000 series, Lytzen Lab, Herlev, Denmark) at 37°C overnight. Bound or adhered bacteria were enumerated by counting under (100x) oil immersion with a microscope (Axioskop microscope). 20 random microscope fields were counted and adhesive cells presented as numbers per 20 fields. All the strains were tested in duplicates and 3 times with Caco-2 cells from three successive passages (8-13 cell passages). Results were graded as 0-10: non adhesive, 11-100: adhesive, >100:strongly adhesive.

2.7 *In vivo studies by pig feeding trials*

Fermented maize dough prepared with two starter cultures *Lb. fermentum* A7-11 and A4-25 (Halm et al., 1996) were fed for a period of 4 weeks to 8 newly weaned 4 weeks old Yorkshire and Danish Landrase, crossbred, castrated male piglets chosen from 4 different sows. Previous studies on the use of starter cultures for kenkey fermentation (Halm et al., 1996) had demonstrated the potential of the strain *Lb. fermentum* (Halm et al., 1996). The fermented maize amounted to 10% of the total feed given to the piglets and contained *Lb. fermentum* in the order of 10^9 cfu/g. At the end of the 4 weeks period the pigs were slaughtered. The content of the small intestine was divided into three parts the upper, middle and lower part. Samples of fermented maize dough and the content of the stomach and various segments of the intestine tract as indicated in Fig 2 were examined for the presences of lactobacilli, determined as colony forming unit (cfu/g), in Universal Beer Agar (UBA: Merck, Darmstadt, Germany) incubated anaerobically at 30°C for 5 d (Halm et al., 1993). Growth of lactobacilli was verified by colony morphology, microscopy and catalase reaction. For the upper segment of the small intestines segments (2) Fig. 2 of 4 piglets 60 representative colonies of lactobacilli, 15 each per piglet were selected. Identification was carried out by the RAPD method described by Hayford and Jakobsen (1998) using arbitrary primers of 9 base pairs (primer 5'-ACGCGCCCT-3') for specific recovery of *Lb. fermentum* strains A7-11 and A4-25

3. Results

3.1 Resistance to pH 2.5

There was no significant growth observed for the isolates from fermented maize dough in MRS pH 2.5 incubated at 37°C for 7 h. However, the 21 isolates were able to survive and 18 showed no apparent loss of viability of cells determined as colony forming units (results not shown). Three strains A7, A31 and F7 showed a decline in cell viability, while the strains H5 and G6 showed an increase but these changes never exceeded a factor of 10 or the original number of cfu/g (results not shown). Except for strains H5 and G6 showing an increase, the OD readings remained constant indicating that cell lysis did not occur (results not shown).

3.2 Resistance to Bile acids

All isolates were resistant to 0.3% bile acids and seemed to have a higher growth capacity in the bile acid environment than in the acidic medium. Results of the viability of cells in bile acid medium is shown in Table 2. Increase in OD were observed in most strains, similarly increases of cell count (cfu/ml) were observed (results not shown). *Lactobacillus fermentum* strains D17, A7 and G20 showed a decline in cell viability determined as cfu/g (results not shown). Compared to the control, growth was delayed for all isolates (results not shown).

3.4 Antimicrobial activity

The results of the inhibitory activity of *Lactobacillus fermentum* strains expressed as the zones of inhibition (diameter in millimeters) against pathogenic bacteria are shown in Table 3. The strains varied in their inhibitory activity. Most the strains were antagonistic against *Shigella flexneri* this accounting for 90% of the strains, 47% inhibited the growth of *Salmonella typhimurium* and 42% inhibited *E. coli*. 19% of the strains inhibited the growth of *Bacillus cereus* and 19% was antagonistic against *L. monocytogenes*. For these experiments MRS with reduced glucose (0.2%) was used. Experiments with normal MRS including 2% glucose, showed strong antagonism (inhibition zones,16-20 mm) for *E. coli, L. monocytogenes* and *Bacillus*

Table 2 Survival and growth of *Lb. fermentum* isolates in Bile acid (0.3% oxgall).

	Optical Density (OD)			
	MRS (0.3% oxgall)		Control (MRS)	
Strains	0 h	7 h	0 h	7 h
B13	0.080	0.182	0.079	0.333
H11	0.078	0.150	0.084	0.776
D17	0.067	0.052	0.075	0.403
G12	0.072	0.214	0.070	0.749
G10	0.061	0.231	0.063	0.498
A7-11	0.150	0.130	0.150	0.750
A31	0.076	0.111	0.065	0.369
A7	0.072	0.093	0.077	0.786
C2	0.060	0.162	0.064	0.520
G20	0.081	0.081	0.071	0.912
I22	0.09	0.138	0.073	0.756
A4-25	0.050	0.100	0.060	0.500
H17	0.082	0.152	0.086	0.460
A28	0.075	0.192	0.089	0.549
H5	0.063	0.162	0.061	0.960
A13	0.065	0.262	0.058	0.508
F5	0.077	0.120	0.083	0.454
F7	0.074	0.213	0.063	0.701
G6	0.071	0.179	0.073	0.762
G11	0.084	0.307	0.080	0.771
C14	0.080	0.152	0.084	0.980

cereus for all strains (results not shown). Similar observation were made for *Salmonella typhimurium* and *Shigella flexneri* but the latter was completely inhibited for all the strains with normal MRS (results not shown). Inhibition experiments were carried out with the supernatant broth of isolates A7, A28, D17 and G11 by the well assay technique. No inhibition was recorded for the supernatant both with or without ph adjustment (6.5-7.0). However inhibition zones were recorded for 10 fold concentrated supernatant with or without pH adjustment for these strains (results not shown).

3.5 *Adhesion experiments*

The ability of strains to adhere to Caco-2 cells are shown in Fig 1. The data was expressed as numbers of bacteria bound per 20 microscope fields. Fig. 1 shows the adhesion of the reference and known probiotic *Lactobacillus plantarum* strain 299 (Johansson et al, 1993) and *Salmonella typhimurium* TM 3931 reported as strongly adhesive. The *Lb. fermentum* strains differed in their ability to adhere to Caco-2 epithelial cells. Compared to known positives strains (*Salmonella typhimurium* TM 3931, *Lactobacillus plantarum* 299) which were all strongly adhesive, strains from maize dough could be rated only as adhesive. Seven strains had the ability and potential to adhere to Caco2-human epithelial cell lines namely B13, G11, A4-25, A7-11, D17, A7, F5.

3.6 *Pig feeding trials*

The content of lactobacilli cfu/g in the stomach and the three sections of the small

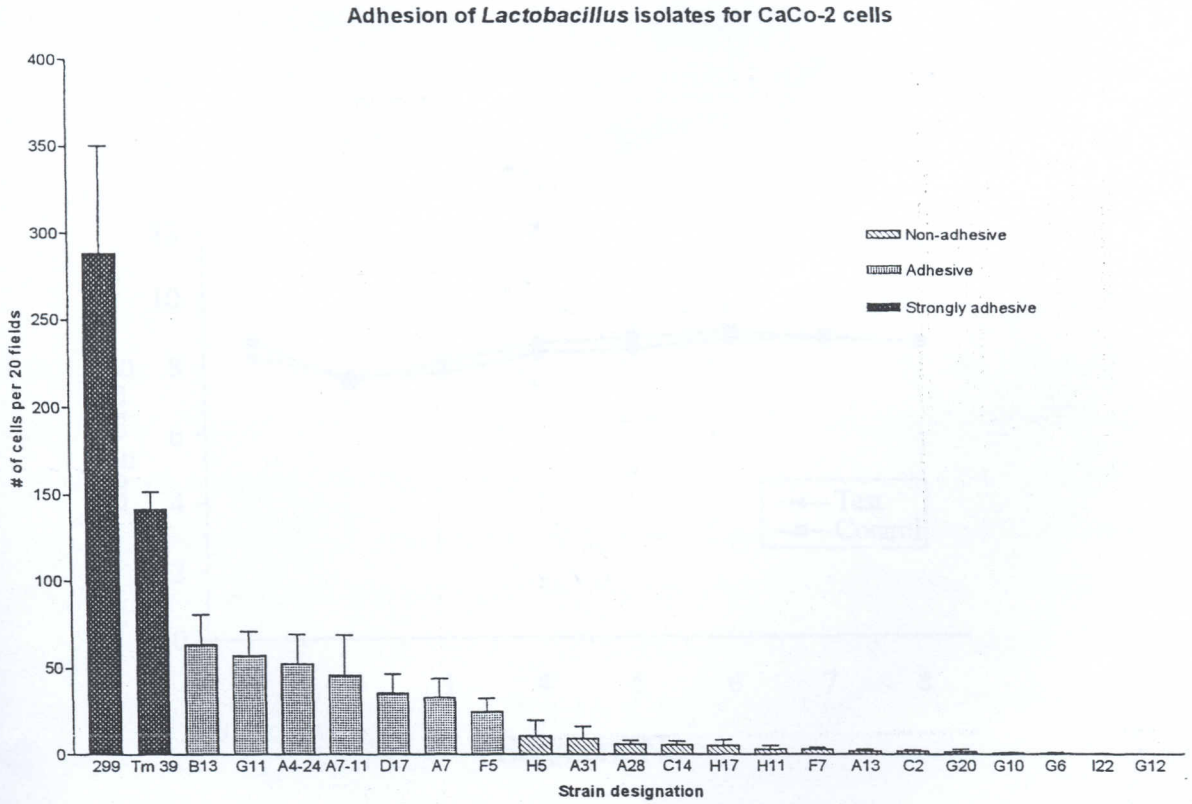

Fig. 1 Adhesion of *Lactobacillus fermentum* isolates from fermented maize dough, and known adhesive *Lactobacillus* species and *Salmonella typhimurium* to Caco-2 cells. The adhesion index is the mean number of cells per 20 microscope field determined in three separate experiments in duplicates. Horizontal bars indicate mean standard error.

intestines of the pigs with their mean standard deviation were 1.8×10^8 (1.11×10^8), 5.16×10^7 (4.71×10^7), 5.70×10^7 (3.77×10^7), 2.12×10^8 (1.43×10^8) cfu/g respectively, from the stomach and the upper, middle and lower section of the small intestines. Fig 2 shows the content of *Lb. fermentum* from the stomach (1), the small intestines (2-4), and caecum (5), colon (6 and 7) and rectum(8). A decrease in the cell numbers occurred in the upper section of the small intestines. Detailed identification by the RAPD method for isolates, 15 per piglets from the upper part of the small intestine showed that they were all identical to the starter culture used to reproduce maize fermentation, as shown in Fig. 3. The figure shows the profiles for the two *Lactobacillus fermentum* starter used and the profiles of some of the isolates from the fermented maize dough. The decrese was followed by a slight increase which was maintained all though the gastrointestinal tract. For all the strains isolated from segment 2 (Fig. 2) profiles were similar to starter culture *Lactobacillus fermentum* A4-25 non of the profiles matched that of the starter *Lactobacillus fermentum* A7-11.

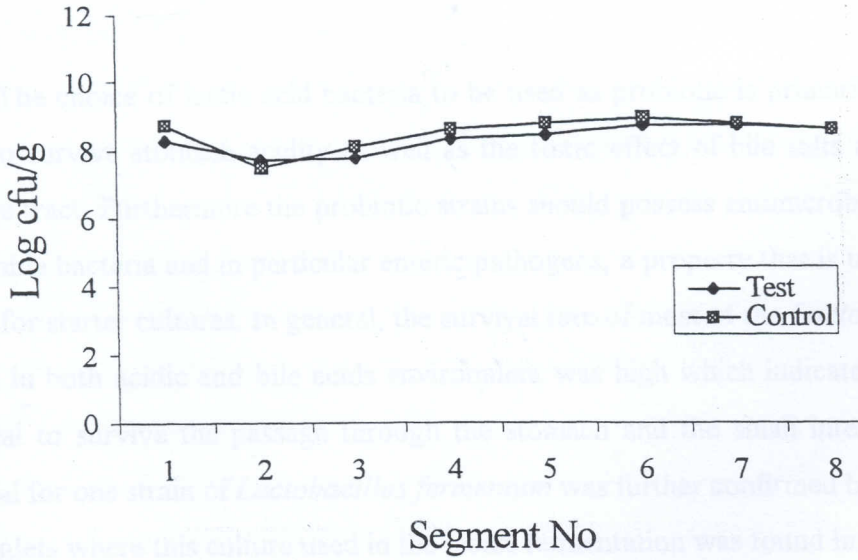

Fig. 2 Density of lactic acid bacteria per gram of gastrointestinal content in various segments of pigs gut. Segment no: stomach (1); small intestines upper part (2) middle part (3) and lower part(4); caecum (5); colon(6,7); rectum (8).

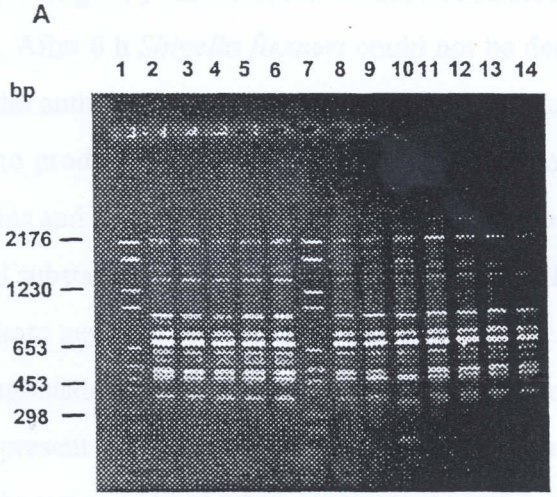

Fig. 3 RAPD profiles of some selected *Lactobacillus fermentum* isolates from maize dough and the two starters *Lactobacillus fermentum* (A4-25, A7-11)used in the fermentation showing two distinct bands at 773 bp and 695 bp typical of *Lactobacillus fermentum* (Hayford et al., 1998) Lanes 2 to 6, (isolates from 0 h fermentation) and lane 8 (steep maize isolate), lanes 9 and 10 (A4-25), A7-11), and lane 11to 14 (isolates from 24 h fermentation), lane 1 and 8: DNA markerVI (Boehringer Mannheim) with molecular weights as follows:2176, 1766, 1230, 1033, 653, 517, 453, 394, 298, 234, and 154 bp.

4. Discussion

The choice of lactic acid bacteria to be used as probiotic is primarily based upon their ability to survive stomach acidity as well as the toxic effect of bile salts and to colonize the digestive tract. Furthermore the probiotic strains should possess antimicrobial activities against undesirable bacteria and in particular enteric pathogens, a property that is useful as a selection criteria for starter cultures. In general, the survival rate of most of the *Lactobacillus fermentum* isolates in both acidic and bile acids environment was high which indicated that they had the potential to survive the passage through the stomach and the small intestines. The survival potential for one strain of *Lactobacillus fermentum* was further confirmed by the *In vivo* studies with piglets where this culture used in the maize fermentation was found in the intestines and in the faeces of the piglet fed with the maize dough fermented with this strain.

The antimicrobial activity of 21 *Lactobacillus fermentum* strains were variable. Some strains had

the potential of producing antimicrobial compounds which could inhibit enteric bacteria and in particularly *Shigella flexneri*. Mensah et al. (1988; 1991) in their study with maize dough also emphasized the fact that *Shigella flexneri* could not survive in the maize dough when inoculated with *Escherichia coli*. After 6 h *Shigella flexneri* could not be detected while *Escherichia coli* survived longer and the antimicrobial activity was still. The different strains of *Lactobacillus fermentum* appeared to produce slightly different antimicrobial compounds due to the multiple inhibition of some strains and the specificity of some strains only for *Shigella flexneri*. However, some strains produced substances that seemed broad spectrum in their activity, and could inhibit the growth of both Gram negative and Gram positive pathogens. But surprisingly with the 21 strains tested the antagonistic activity observed against *Bacillus cereus* and *L. monocytogenes* was low. Non of the present cultures were shown to excrete antimicrobial compounds into the liquid medium under the test conditions when supernatant broth were tested. Similar results were obtained by Geis et al. (1992) who tested 93 strains of *Streptococcus lactis* subsp. *cremoris* (*Lactococcus lactis* subsp. *cremoris*) and found that 36 strains exhibited antagonistic effects in agar diffusion tests, but only one of them produced antimicrobial substance in the liquid medium. Herrero et al (1996) suggested that the antimicrobial compounds produced by some strains maybe unstable or degraded by proteases. In our study however, concentrated supernatant in several cases showed antimicrobial effect indicating that the concentration of the antimicrobial substances may play a part in the antagonistic activity (Schillinger and Holzapfel 1990). However, this remains to be fully elucidated for the present cultures.

Olsen et al. (1995) in their studies found all the *Lactobacillus fermentum* isolates from fermented maize dough to be inhibitory toward both Gram negative and Gram positive bacteria including *Escherichia coli* and *Staphylococcus* spp. They concluded that the mechanism of these antimicrobial effect were dominated by acid production and formation of compounds sensitive to proteolytic enzymes indicating the possibility of production of bacteriocin

The Caco-2 cells lines as a model for probiotic adherence studies and has been successfully used by other workers in similar studies (Elo et al., 1991; Chauviere et al., 1992; Sarem et al., 1996; Tuomola and Salminen., 1998). The ability of *Lactobacillus fermentum* strains to adhere to the Caco-2 cells varied considerably between strains, which showed that the adhesive properties are not a universal feature of the species. The adhesive property of lactic acid bacteria has been suggested to be related to the origin of the organism, whereby, organism isolated from the gut

tend to be more highly adhesive than strains isolated from plant or food material (Chauviere et al., 1992). The mechanism of adhesion is not fully known and although we did not investigate this in our study various school of thought indicate that the process is mediated by a proteinaceous material which is excreted into the spent broth culture media and others report adhesions as being calcium dependant (Busscher et al., 1987; Chauviere et al., 1992; Sarem et al., 1996). Nonetheless, the presences of starter cultures in the piglet intestines with the *in vivo* studies indicate that some strains can survive the passage of the stomach and transient colonization of the gut by these strains is likely to take place.

The data reported here indicate that a rich source of strain variability exists in the microbiota present in fermented maize dough in which commercial staters are not used. The possibilities of selecting strains with important probiotic characteristics like antimicrobial activities pH and bile tolerance and adhesion to epithelial cells have been emphasized.

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