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Full Length Research Paper

Phenotypic diversity of lactic acid bacteria isolated from *Massa*, a fermented maize dough

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A total of thirty lactic acid bacteria belonging to *Lactobacillus fermentum, Lactobacillus plantarum, Pediococcus acidilactici, Lactococcus lactis and Leuconostoc mesenteroides* were isolated from the various fermenting stages of white maize gotten from five western states of Nigeria namely Oyo (Ibadan), Lagos, Ondo (Akure), Ogun (Abeokuta) and Ekiti states for *Massa* production and were subjected to physiological tests. *L. fermentum, L. plantarum, P. acidilactici* had high survival rates of between 4.00 – 5.23 $\log_{10}$ cfu/ml at a very low pH value of 2. *L. lactis* produced the highest quantity of lactic acid (8.00 g/l) while *L. plantarum* produced the highest quantity of hydrogen peroxide (1.68 g/l). A high percentage of phytic acid (5.52 - 6.47%) was produced by *L. fermentum, L. plantarum* and *P. acidilactici*. *L. fermentum, L. plantarum* and *P. acidilactici* grew well at both low and high temperatures of 10 and 45°C, respectively. All the isolates survived freezing and spray drying conditions with *L. plantarum and P. acidilactici* having the highest survival rate of 5.62 $\log_{10}$ cfu/ml during freezing while *L. fermentum* had the highest survival rate of 5.45 $\log_{10}$ cfu/ml during spray drying. All the isolates produced protease and amylase enzyme but did not produce cellulase enzyme. These results showed that the phenotypic diversity of the lactic acid bacteria flora during maize grains fermentation depended on the variety of maize and the sampling location.

Key words: Lactic acid bacteria (LAB), phenotypic, diversity, *Massa*.

INTRODUCTION

Maize is a staple food consumed as daily diets in many parts of the world. Various food products have been produced from maize such as *mahewu, pozol, ogi, banku, Kenkey, koko* etc. One of the popularly eaten food products gotten from maize that has not been microbiologically evaluated extensively is *Massa*.

Massa is a traditional Nigerian snack produced from fermented maize dough. It is a product of *Zea mays* produced from the white variety of maize (Oyeyiola, 1990). It is consumed extensively in the Northern and southern parts of Nigeria. *Massa* tastes sour due to its high acid content therefore it is eaten with granulated sugar or honey. It's a popularly known and eaten food in Nigeria but because of the mode of its production, in which standards are not available to ensure that the products is of the best quality in all ramifications, there is a variation to the taste, flavour, sourness or acidity and the microbial load.

For traditional fermentation of *Massa,* the maize grains were soaked in water for 2 - 3 days after which the water were decanted and washed with fresh clean water followed by wet milling without much water. The run - off water from the milling mash was collected in a separate bowl and allowed to settle. The slurry was boiled for 20 min and then allowed to cool. The mash was then mixed with the maize slurry and covered with a tray for fermentation to occur for 12 - 24 h; the fermented sour dough was rolled by hand into balls and fried in vegetable oil for 5 - 10 min using earthen ware pot (Oyeyiola, 1990).

Odunfa (1985) stated that fermentation processes occur by chance inoculation and are usually initiated by mixed microflora. Though according to Oyewole and Odunfa (1990), non - lactic acid micro organisms are eliminated with increasing acid production in the

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fermenting substrate or medium. Ampe and Omar (2000) isolated a wide variety of microorganisms including fungi, yeasts, lactic acid bacteria and non- lactic acid bacteria from fermented maize dough. Fermented maize dough is used for making a variety of product and the process includes four different microbiological environments; the maize grains, steeping of the maize grains in water for 24 h, milling of the steeped maize and preparation of the fresh dough and fermentation of the dough for about 48 h (Olsen et al., 1995).

During steeping and fermentation of maize, the microbial flora consist of a mixed population of lactic acid bacteria, gram - positive, catalase positive and gram - negative bacteria, yeast and moulds, but the flora is rapidly changed towards a uniform population of heterofermentative lactic acid bacteria mainly *Lactobacillus fermentum* and *Lactobacillus reuteri* (Halm et al., 1993; Hounhouigan et al.,1993). Other lactic acid bacteria associated with fermented maize are *Lactobacillus plantarum* (Akinrele, 1970; Fields et al., 1981), *Pediococcus pentosaceus* and *Pediococcus acidilactici* (Halm et al., 1993). Apart from lactic acid bacteria, yeast also form an important part of the microbial flora in fermented maize (Akinrele, 1970; Halm et al., 1993; Hounhouigan et al., 1993; Jesperson et al., 1994).

Lactic acid bacteria are a special group of bacteria which are classified as Generally Recognized As Safe (GRAS) (Phumathon, 1999). Aquirre and Collins (1993) also described lactic acid bacteria as a broad group of Gram - positive, catalase negative, non-sporing rods and cocci usually non-motile that utilize carbohydrate fermentation and form lactic acid as the major end product. However, Muller (2000) described lactic acid bacteria as a group of bacteria united by a constellation of morphological, metabolic and physiological characteristics.

Today, lactic acid bacteria, with certain importance in foods can be assigned to the genera *Camobacterium*, *Vagococcus, Enterococcus, Aerococcus, Allolococcus, Tetragenococcus, Lactococcus, Streptococcus, Weissella, Leuconostoc, Lactobacillus* and *Pediococcus* (Vandamme, 1994).

The various lactic acid implicated in cereal based African fermented foods include *L. plantarum* and *L. fermentum* from *ogi* (Onyekwere et al., 1989). Ihekoronye and Ngoddy (1985) gave the following bacteria as also being some of the most important species in the lactic acid bacteria group: *Lactobacillus lactis, L. bulgaricus, L. plantarum, L. acidophilus, L. delbrueckii, L. mesenteroides and L. dextranicum.*

L. fermentum and *P. acidilactici* also formed major isolates during natural lactic acid fermentation of maize grains to produce corn meal (Fields et al., 1981). *L. fermentum* has frequently been reported as one of the major lactic acid bacteria isolated from African fermented cereal dough (Hounhouigan et al., 1993; Halm et al., 1996; Hamad et al., 1997) and its importance in Ghanaian fermented maize dough was recently highlighted (Hayford et al., 1999).

Lactic acid bacteria (LAB) are commonly involved in the fermentation of carbohydrate based substrates (Beukes et al., 2001). The major metabolic products are organic acids, lactic acid, bacteriocins and hydrogen peroxide. Lactic acid fermentation of food has been found to reduce the risk of having pathogenic microorganisms grow in the food (Sahlin, 1999).

LAB contributes to the taste and texture of fermented products and inhibits food spoilage bacteria by producing growth - inhibiting substances (bacteriocins) and large amounts of lactic acid. Many LAB benefit human and animal health whereas others spoil beer, wine and processed meats.

Lactobacillus species in fermented foods has also been considered as probiotics but has also been used as a live microbial feed supplement, which is beneficial to the host animal through improving its intestinal microbial balance (Steinkrause, 1995). *L. acidophilus* has also been implicated for its use as probiotics. However, a wide range of *lactobacilli* has been used in probiotic preparations. These include *Lactobacillus delbreuckii* subsp. *bulgaricus*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus cellobiosus, L. lactis*, *L. fermentum*, *L. plantarum* and *L. reuteri* (Steinkrause, 1995; Vinderola et al., 2002).

Lactobacillus and *Streptococcus faecium* are beneficial microorganisms, which have been proven to replenish essential microflora and decrease the incidence of gastrointestinal disorders. Beneficial bacteria, especially *lactobacillus* sp. can produce anti- microbial substances, which have been observed to inhibit the growth of some pathogenic micro organisms.

Lactic acid bacteria (LAB) are the most important bacteria used in food fermentation. The group of lactic acid bacteria (LAB) occupies a central role in these processes and has a long and safe history of application and consumption in the production of fermented foods and beverages (Caplice and Fitzgerald, 1999; Ray, 1992; Wood, 1997; Wood and Holzapfel, 1995). They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes are of importance. In this way they enhance shelf life and microbial safety, improve texture and contribute to the pleasant sensory profile of the end product. They can be isolated from the respiratory, intestinal and genital tracts of humans and animals and from plants.

In this study we investigated the phenotypic characteristics of lactic acid bacteria isolated from the different samples of fermented white maize dough used for *Massa* production so as to ascertain if the characteristics exhibited by the isolates from the different maize samples differed.

MATERIALS AND METHODS

Isolation and characterization of lactic acid bacteria during maize fermentation for *Massa* production

The maize grains were soaked in water for 48 h for fermentation to occur. The maize samples were taken at 12, 24 and 48 h and grinded using a blender. 1 g of the ground samples was taken and was added to 9.0 ml of distilled water in a test tube and diluted serially. 0.1 ml of the aliquots of 10^{-4} and 10^{-5} dilutions were aseptically dispensed onto sterile plates and MRS (De Man Rogosa Sharpe) agar with adjusted pH 5.5 was poured onto it and allowed to set. The plates were incubated at 37°C for 48 h under anaerobic conditions. Discrete colonies were streaked onto fresh agar to obtain pure cultures of the different isolates. The pure colonies were characterized using cultural characteristic and biochemical tests.

Isolates were maintained on MRS agar slants and stored at 4°C for further tests. Cultural characteristics were based on their luminescence, shape, elevation, colonial edge, consistency, surface and pigmentation. Biochemical tests performed on the bacterial isolates included gram staining, spore staining, catalase activity, oxidase test, gelatine hydrolysis, starch hydrolysis, indole production, sugar utilization, methyl red test, voges proskauer test, production of ammonia from arginine, hydrogen sulphide production and motility test.

Determination of visible growth and viable count of LAB isolates at 10, 15 and 45°C

MRS broth from Oxoid laboratories Inc. was used to carry out this test. 5.5 g of the broth was dispensed into 100 ml of clean, distilled water and homogenized in the water bath. 5 ml of the broth was dispensed into super bottles and sterilized in the autoclave at 121°C for 15 min. The broth was allowed to cool and aseptically they were inoculated with the test isolates and incubated at 10, 15 and 45ºC for 48 h. After incubation, the broth was examined visually with the control tubes for each temperature in which no organism was used to inoculate. The broth with visible growth appeared turbid as compared with the control that was very clear. For each of the broth incubated at different temperatures, they were serially diluted to a dilution factor of 10^{-4}. 1 ml of which was taken and dispensed into sterile plates and then the media poured onto it, swirled gently and allowed to set. After which it was incubated at 37°C anaerobically for 48 h and the distinct and viable colonies formed were counted.

Acid tolerance test determination

The method of Conway et al. (1987) was employed. The cultures were grown in MRS broth and incubated anaerobically at 37°C overnight. Thereafter, the cultures were centrifuged at 2000 g for 10 min at 4°C and the pellets were washed twice in sterile phosphate buffered saline (pH 7) and then re- suspended in 10 ml of phosphate buffered saline. The concentration of the suspension was determined by examining the turbidity of the suspension using Jenway 6051 colorimeter. The number of lactic acid bacteria per ml was determined by plating out 10 fold serial dilution of the suspension on MRS agar. The titre was expressed as colony forming unit per ml for each lactic acid bacterial strain. 0.1 ml of culture suspension with a concentration of 4.5 x 10^5 cfu/ml was added separately into a series of tubes containing 2 ml of sterile phosphate buffered saline at various pH values (2 - 5). Hydrochloric acid (0.1 ml) was used to adjust the pH of the phosphate buffered saline as required. The tubes were incubated for 0, 1, 2 and 3 h. After the incubation period, 0.1 ml from each tube was cultured on MRS agar plates and incubated at 37°C for 48 h and followed by the determination of viable count.

Quantitative determination of hydrogen peroxide and lactic acid

Lactic acid determination

The method of Ogunbanwo et al. (2003) was employed. The isolates were grown in MRS broth for 48 h at 37°C and centrifuged for 15 min. After centrifugation, the supernatant was further purified of any left over cells by filtration using Whatman No. 1 filter paper.

25 ml of the filtrate was dispensed into a conical flask and 3 drops of phenolphthalein indicator was added. From a burette, 0.1 ml NaOH was slowly added to the samples until a pink colour appeared. Each ml of 0.1 ml NaOH is equivalent to 90.08 mg of lactic acid (A.O.A.C, 1990). Thus titratable acidity is expresses as:

$$\text{Titratable acidity} = \frac{\text{ml NaOH} \times \text{N NaOH} \times \text{M.E}}{\text{Volume of sample used}}$$

ml NaOH = Volume of NaOH used; N NaOH = concentration of NaOH used; M.E = Equivalence factor.

Hydrogen peroxide determination

The isolates were grown in MRS broth for 48 h at 37°C and centrifuged at 3000 g for 15 min. After centrifugation, the supernatant was filtered using Whatman no 1 filter paper. 20 ml of diluted sulfuric acid was added to 25 ml of the supernatant fluid of the test organisms. Titration was carried out with 0.1 M potassium permanganate. Each ml of 0.1 M potassium permanganate is equivalent to 1.79 mg of hydrogen peroxide. Solution and decolorization of the sample was regarded as the endpoint (A.O.A.C, 1990). The concentration of H_2O_2 produced by isolates is calculated thus,

$$H_2O_2 \text{ concentration} = \frac{\text{ml } KmnO_4 \times \text{N } KmnO_4 \text{ M.E} \times 100}{\text{ml } H_2SO_4 \times \text{volume of sample}}$$

ml $KmnO_4$ = volume of $KmnO_4$ used, N $KmnO_4$ = concentration of $KmnO_4$ used,
M.E = Equivalence factor, ml H_2SO_4 = volume of H_2SO_4 added to the sample.

Qualitative determination of enzyme production by the LAB isolates

Qualitative assay for the determination of the production of amylases, proteases and cellulases on agar medium was done as described by Fawole and Osho (1986).

Amylase production

Starch agar was used for this test and the method of Fawole and Osho (1986) was employed. The pH of the medium was adjusted to 7.0. The medium was sterilized and then poured into plates and allowed to set. The medium was then streaked with the isolate and incubated at 35°C for 48 h. After incubation, the plates were flooded with iodine. A positive result indicating the production of amylase by

isolates was indicated by a colourless zone of clearance around the growth while a negative test retains the colour of the iodine.

Protease production

Milk agar was used for this test and was prepared using the method of Ogbulie et al. (1998). The pH was adjusted to 7.2 before sterilizing. After sterilization of the medium, the medium was then poured into plates and allowed to set. The medium was then streaked with the isolate and incubated at 35°C for 6 days. A positive test is indicated by a clear zone around the streak of the isolate on the medium.

Cellulase production

The method described by Ogbulie et al. (1998) was employed. Cellulose agar was used for this test. After sterilization of the medium, it was then poured into plates and allowed to set. The medium was streaked with the isolate and incubated at 35°C for 6 days after which the plates were examined for zones of clearance.

Quantitative determination of phytic acid produced by the LAB isolates

The method of Maga (1982) was employed. 2 g of each inoculated broth sample was weighed into a 250 ml conical flask. 100 ml of 2% hydrochloric acid was added to soak each sample in the conical flask for 3 h. This was filtered through a double layer of hardened filter paper. 50 ml of each filterate was placed in 250 ml conical flask 107 ml of distilled water was added in each case to give proper acidity. 10 ml of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was titrated with slightly brownish yellow that persisted for 5 minutes. The % phytic acid was calculated using the formula,

$$\% \text{ phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 3.55 \times 100}{\text{Weight of sample}}$$

Determination of the cell viability of LAB isolates after spray drying and freezing

The modification of the method by Brian and Etzel (1997) was employed. The LAB isolates were cultured in MRS broth for 48 h at 37°C.

Freezing effect

The inoculated broth was centrifuged at 2000 g for 10 min. the supernatant was discarded and the residue containing the cells was suspended in sterile phosphate buffer and used to wash the cells by centrifuging and then the supernatant was discarded off. This was done three times to get the cells. The cells were frozen in the freezer at a temperature of -20°C overnight. After freezing, the frozen broth was dissolved in a hot water bath after which it was re-suspended in 9 ml of sterile distilled water and then serially diluted to four dilutions (10^{-4}). 1 ml of the fourth dilution was plated using the pour plate method and MRS agar medium was used. The plates were then incubated at 37°C for 48 to 72 h in anaerobic jars and the colony forming units were determined.

Spray drying effect

The inoculated broth was centrifuged at 2000 g for 10 min. The supernatant was discarded and the residue containing the cells was suspended in sterile phosphate buffer and used to wash the cells by centrifuging and then the supernatant was discarded off. This was done three times to get the cells. The cells were now exposed to a temperature of 65°C from a vacuum dryer during the process of spray drying. 1 g of the spray dried powder was rehydrated with 3 ml of autoclaved de-ionized water. The rehydrated samples were kept in ice bath for 30 min to allow complete dissolution. The suspension was serially diluted to four dilutions (10^{-4}). 1 ml of 10^{-4} dilution was plated using the pour plate method. Colony forming units were determined after incubation for 48 to 72 h at 37°C in anaerobic jars.

RESULTS

Thirty strains of lactic acid bacteria were isolated from five different maize samples that were utilized for *Massa* production and thus the phenotypic characteristics of the isolates were studied in this work.

Table 1 shows the effect of different temperatures on the LAB isolates. At 10°C, *L. mesenteroides and L. plantarum* had the highest viable count of 5.51 ($\log_{10}$cfu/ml). At 15°C, *P. acidilactici* had the highest viable count of 6.92 ($\log_{10}$cfu/ml). However at 45°C, there was a marked decline in the viable count of all the LAB isolates. *L. fermentum* had the highest viable count of 5.85 ($\log_{10}$ cfu/ml) at 45°C.

The survival rate of the LAB isolates at different pH levels is shown in Table 2. The viable count of the LAB isolates increased as the pH increased. At pH 2, *L. plantarum* had the highest viable counts of 5.23 ($\log_{10}$ cfu/ml) while some strains of the LAB isolates did not grow at all but at the pH 5, *L. fermentum* had the highest viable counts of 6.01 ($\log_{10}$ cfu/ml). However, it was noted that as the acidity decreases, the viable counts for all the LAB isolates increased.

Five species of LAB were studied for quantitative production of lactic acid (Table 3). *L. lactis* produced the highest quantity of lactic acid (8.00 g/l) while *L. fermentum* produced the lowest quantity of lactic acid (0.55 g/l). However, all the LAB isolates studied produced lactic acid to varying degrees.

The quantity of hydrogen peroxide produced by all the LAB isolates was studied. The highest quantity of hydrogen peroxide produced by all the LAB isolates was studied. The highest quantity of hydrogen peroxide was produced by *L. plantarum* (1.68 mg/ml) while the lowest quantity was produced by *P. acidilactici* (0.36 mg/ml) (Table 3).

The LAB isolates produced phytic acid, with *L. plantarum* producing the highest quantity of 6.30% while *L. lactis* and *L. mesenteroides* had the lowest quantity of 1.89% phytic acid each (Table 3).

Table 4 shows the enzyme production by the LAB isolates. All the LAB isolates studied did not produce cellulose enzyme *while P. acidilactici, L. plantarum* and *L.*

Table 1. Viable counts of LAB isolates at different temperatures (log_{10}cfu/ml).

Isolates	10°C	15°C	45°C
P. acidilactici MIb1	5.42	5.51	5.72
L. fermentum MIb2	5.37	5.45	5.62
P. acidilactici MIb3	5.18	5.96	5.48
L. plantarum MIb4	5.34	6.09	5.83
L. lactis MIb5	5.20	6.06	5.75
*L. mesenteroides*MIb6	5.30	5.90	5.62
P. acidilactici MLa1	5.15	5.87	5.58
L. plantarum MLa2	5.38	5.95	5.78
L. fermentum MLa3	5.25	5.94	5.60
L. lactis MLa4	5.08	6.00	5.68
L. lactis MLa5	5.45	5.86	5.72
L. mesenteroides MLa6	5.51	6.08	5.79
P. acidilactici MAk1	4.70	5.90	5.40
L. fermentum MAk2	5.45	6.03	5.70
L. plantarum MAk3	5.26	5.93	5.72
P. acidilactici MAk4	5.08	5.99	5.62
L. fermentum MAk5	5.60	5.83	5.85
L. fermentum MAb1	5.48	5.87	5.76
L. plantarum MAb2	5.30	6.92	5.66
P. acidilactici MAb3	5.41	6.03	5.78
P. acidilactici MAb4	4.78	6.00	5.45
L. lactis MAb5	5.25	5.86	5.72
P. acidilactici MAb6	5.48	6.08	5.83
L. plantarum ME1	5.51	5.95	5.68
L. mesenteroides ME2	5.45	5.85	5.72
L. fermentum ME3	5.30	5.89	5.68
L. plantarum ME4	5.30	5.82	5.81
P. acidilactici ME5	5.08	6.09	5.58
L. lactis ME6	5.41	5.99	5.67
L. plantarum ME7	5.34	5.91	5.83

Table 2. Survival of the LAB at various pH levels (log_{10}cfu/ml).

Isolates	pH			
	2	3	4	5
P. acidilactici MIb1	5.04	5.89	5.95	5.99
L. fermentum MIb2	0.00	4.60	5.18	5.48
P. acidilactici MIb3	0.00	5.81	5.94	5.98
L. plantarum MIb4	4.30	5.36	5.66	5.94
L. lactis MIb5	0.00	4.85	5.15	5.68
*L. mesenteroides*MIb6	4.60	5.41	5.72	5.89
P. acidilactici MLa1	4.69	4.90	5.56	5.89
L. plantarum MLa2	5.23	5.32	5.45	5.53
L. fermentum MLa3	4.00	4.69	5.26	5.57
L. lactis MLa4	0.00	4.69	5.75	5.86
L. lactis MLa5	4.69	4.90	5.62	5.83
L. mesenteroides MLa6	0.00	5.08	5.69	5.72
P. acidilactici MAk1	0.00	5.00	5.32	5.60
L. fermentum MAk2	0.00	5.08	5.81	5.93
L. plantarum MAk3	0.00	5.65	5.38	5.84
P. acidilactici MAk4	4.30	5.08	5.54	5.86
L. fermentum MAk5	0.00	5.04	5.90	5.97
L. fermentum MAb1	4.48	5.78	5.54	5.86
L. plantarum MAb2	0.00	5.00	5.68	5.77
P. acidilactici MAb3	0.00	4.78	5.84	5.91
P. acidilactici MAb4	4.30	5.49	5.57	5.72
L. lactis MAb5	0.00	5.00	5.59	5.64
P. acidilactici MAb6	5.15	4.78	5.59	5.72
L. plantarum ME1	4.30	5.49	5.48	5.62
L. mesenteroides ME2	0.00	5.00	5.89	5.97
L. fermentum ME3	4.00	5.84	5.83	6.01
L. plantarum ME4	0.00	4.30	5.72	5.89
P. acidilactici ME5	4.26	5.08	5.26	5.56
L. lactis ME6	0.00	4.48	5.45	5.62
L. plantarum ME7	4.48	4.69	5.36	5.76

lactis produced amylase enzyme but all the other LAB isolates did not produce amylase enzyme. However, most of the LAB isolates produced protease enzyme.

The ability of the LAB isolates to retain their cell viability when subjected to freezing and spray drying at -20 and 65°C respectively is shown in Table 5.

DISCUSSION

Lactic acid bacteria were isolated from traditionally fermented maize gruels from five different western states of Nigeria and were identified as *P. acidilactici, L. plantarum, L. fermentum, L. lactis* and *Leuconostoc mesenteroides.* This conforms to the work of Oyeyiola (1990) and Muller (2000) who reported the dominance of these organisms in fermented cereals especially fermented maize.

The viable counts of LAB during the maize grains fermentation increased with the time of fermentation for all the maize samples collected from the five different states in Nigeria. This result shows that as fermentation progresses, the LAB population increases and thus aiding the rapid fermentation of the maize grains. This agrees with the work of Oyeyiola (1990).

The increase in production of lactic acid with time has been attributed to lowered pH, which permits the growth of LAB to the detriment of the competing organisms (Kandler and Weiss, 1986). *L. lactis* isolated in the lactic industry is still being studied exhaustively. The major product of fermentation is lactic acid; a compound with a high commercial value, with applications in the food, cosmetic, medical, and pharmaceutical industries (Boonmee et al., 2003). The growth of *L. lactis* is greatly influenced by the temperature and it has only been studied in a few cases. Akerberg et al. (1998), found out

Table 3. Quantity of lactic acid, hydrogen peroxide and phytic acid produced by LAB isolates.

Isolates	Quantity of lactic acid (g/l)	Quantity of hydrogen peroxide (mg/ml)	Quantity of phytic acid (%)
P. acidilactici MIb1	0.81	1.43	5.52
L. fermentum MIb2	1.62	0.91	4.70
P. acidilactici MIb3	2.16	0.86	4.04
L. plantarum MIb4	1.62	0.77	3.91
L. lactis MIb5	2.50	0.64	1.89
*L. mesenteroides*MIb6	1.48	0.91	5.52
P. acidilactici MLa1	0.76	0.91	2.80
L. plantarum MLa2	0.47	0.47	1.89
L. fermentum MLa3	1.59	0.50	5.68
L. lactis MLa4	3.00	0.45	5.07
L. lactis MLa5	4.90	0.54	2.27
L. mesenteroides MLa6	1.54	0.47	1.89
P. acidilactici MAk1	1.17	0.57	4.74
L. fermentum MAk2	1.55	0.63	5.23
L. plantarum MAk3	1.53	0.55	6.30
P. acidilactici MAk4	1.73	0.81	4.61
L. fermentum MAk5	1.15	1.00	2.35
L. fermentum MAb1	0.55	0.68	2.35
L. plantarum MAb2	1.30	0.82	5.60
P. acidilactici MAb3	1.87	0.72	2.88
P. acidilactici MAb4	1.55	1.55	5.15
L. lactis MAb5	3.20	0.88	5.81
P. acidilactici MAb6	1.01	1.59	3.83
L. plantarum ME1	0.57	1.68	6.14
L. mesenteroides ME2	0.85	1.00	3.79
L. fermentum ME3	0.78	0.61	6.47
L. plantarum ME4	1.64	0.44	3.21
P. acidilactici ME5	1.37	0.36	2.27
L. lactis ME6	8.00	0.57	3.62
L. plantarum ME7	1.08	0.41	2.84

that 33.5°C is the temperature which *L. lactis* produces the biggest quantity of lactic acid from glucose. The influence of pH on various characteristics has been well studied; the optimum pH established for growth and product formation is around 6.0 (Bibal et al., 1988; Parente et al., 1994; Akerberg et al., 1998).

The effect of temperature on LAB isolates indicates that at temperatures of 10 and 15°C all the isolates grew well with higher viable counts at 15°C as compared to 10°C while there was a decline in the viable count of LAB at 45°C. This shows that LAB grows more at low temperatures as compared to high temperatures. However, *L. fermentum* grow well at 10 and 45°C which agrees with the findings of Prescott et al. (2002). Most lactic acid bacteria work best at temperatures of 18 to 22°C. The *Leuconostoc* species which initiate fermentation have an optimum of 18 to 22°C. Temperatures above 22°C, favour the *lactobacillus* species (FAO, 1998).

The survival rates of the LAB at various pH levels showed that most of the LAB isolates were tolerant to acidic condition. Generally, survival of the LAB isolates was low at pH 2, moderate at pH 3 and 4, and best at pH 5. *L. plantarum* and *L. lactis* had high survival rates when compared to other LAB species in this study. This could be as a result of the ability of these organisms to produce high quantity of organic acid and the ability to live within the acidic environment.

Lactic acid production by the LAB isolates varied from specie to specie, however *L. lactis* produced the highest quantity of lactic acid. The ability to produce high quantity of lactic acid is grossly dependent on the ability of the producer organisms to utilize carbon and nitrogen source of the medium (Suma et al., 1999). *L. fermentum* produced low quantity of lactic acid. However, the low concentration of lactic acid produced by *L. fermentum* might be attributed to inadequate utilization of carbon and nitrogen source in the medium.

Table 4. Enzyme production by LAB isolates.

Isolates	Amylase	Protease	Cellulase
P. acidilactici MIb1	+	+	+
L. fermentum MIb2	-	+	-
P. acidilactici MIb3	+	+	-
L. plantarum MIb4	-	+	-
L. lactis MIb5	-	-	-
*L. mesenteroides*MIb6	-	+	-
P. acidilactici MLa1	-	-	-
L. plantarum MLa2	+	-	-
L. fermentum MLa3	-	+	-
L. lactis MLa4	+	+	-
L. lactis MLa5	-	+	-
L. mesenteroides MLa6	-	+	-
P. acidilactici MAk1	-	+	-
L. fermentum MAk2	-	+	-
L. plantarum MAk3	+	-	-
P. acidilactici MAk4	-	+	-
L. fermentum MAk5	-	-	-
L. fermentum MAb1	-	-	-
L. plantarum MAb2	+	-	-
P. acidilactici MAb3	-	+	-
P. acidilactici MAb4	-	+	-
L. lactis MAb5	-	+	-
P. acidilactici MAb6	-	+	-
L. plantarum ME1	-	-	-
L. mesenteroides ME2	-	+	-
L. fermentum ME3	-	-	-
L. plantarum ME4	-	-	-
P. acidilactici ME5	-	+	-
L. lactis ME6	-	-	-
L. plantarum ME7	-	+	-

- = Enzyme was not produced by LAB isolates, + = Enzyme was produced by LAB isolates.

Table 5. Effect of freezing and spray drying on the viability of LAB cells (log_{10} cfu/ml).

Isolates	Frozen (-20°C)	Spray dried (65°C)
P. acidilactici MIb1	5.30	5.08
L. fermentum MIb2	5.49	5.45
P. acidilactici MIb3	5.20	5.00
L. plantarum MIb4	5.62	5.32
L. lactis MIb5	5.08	5.41
*L. mesenteroides*MIb6	5.20	5.00
P. acidilactici MLa1	5.56	5.08
L. plantarum MLa2	5.34	5.36
L. fermentum MLa3	5.60	5.18
L. lactis MLa4	5.56	5.32
L. lactis MLa5	5.15	5.26
L. mesenteroides MLa6	5.41	4.90
P. acidilactici MAk1	5.48	5.18
L. fermentum MAk2	5.51	5.43
L. plantarum MAk3	5.41	5.20

Table 5 Contd.

P. acidilactici MAk4	5.45	5.30
L. fermentum MAk5	5.43	5.20
L. fermentum MAb1	5.56	5.20
L. plantarum MAb2	5.49	5.26
P. acidilactici MAb3	5.62	5.08
P. acidilactici MAb4	5.43	5.26
L. lactis MAb5	5.15	5.30
P. acidilactici MAb6	5.49	4.90
L. plantarum ME1	5.30	5.40
L. mesenteroides ME2	5.45	5.04
L. fermentum ME3	5.48	5.15
L. plantarum ME4	5.53	5.20
P. acidilactici ME5	5.45	5.30
L. lactis ME6	5.28	5.08
L. plantarum ME7	5.20	5.28

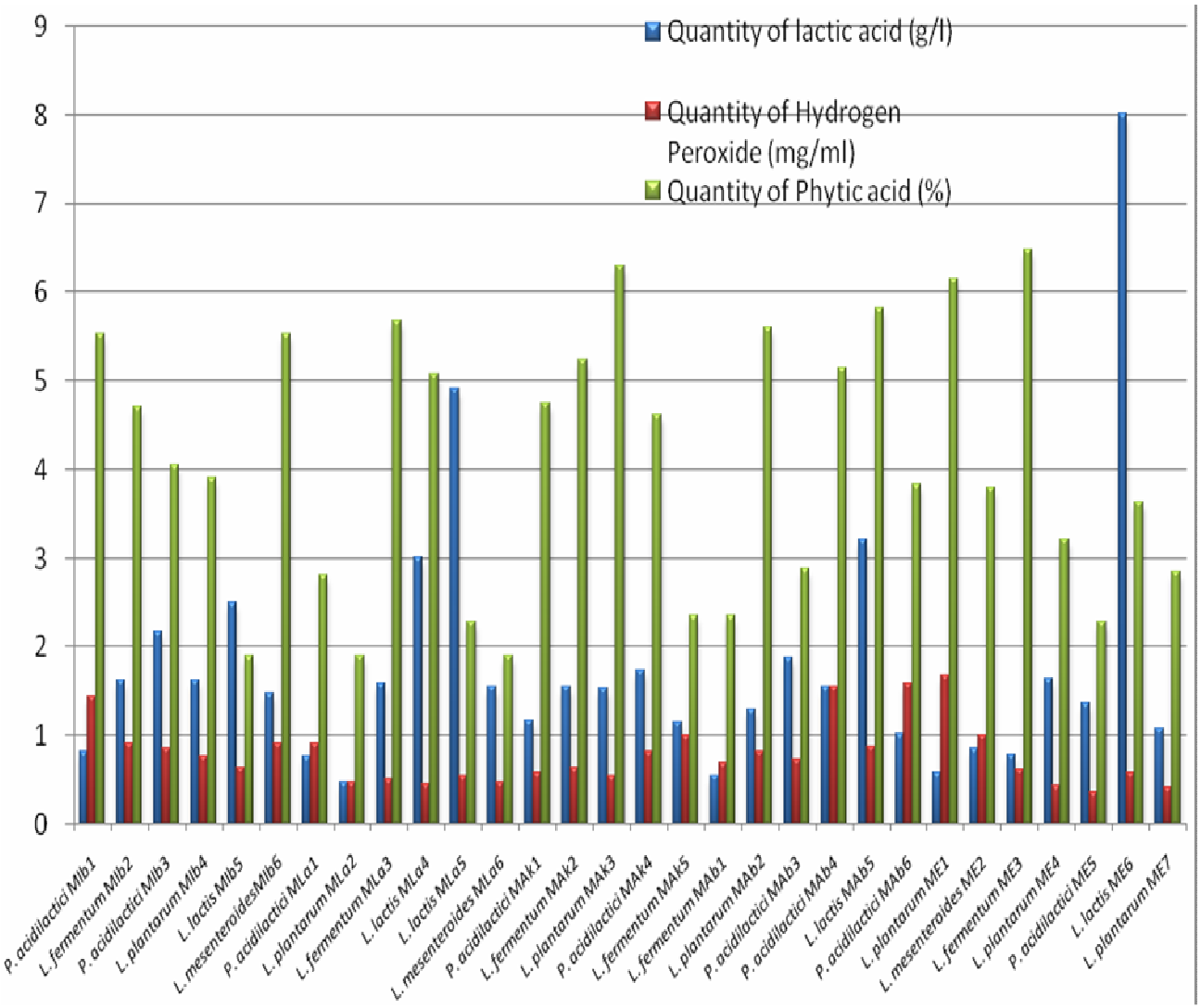

Figure 1. A chart showing the production of lactic acid, hydrogen peroxide and phytic acid produced from LAB.

L. plantarum produced the highest quantity of hydrogen peroxide, this agrees with Prescott et al. (2002) who reported that L. plantarum uses manganese ions instead of superoxide dismutase to destroy the superoxide radical in order to produce hydrogen peroxide. Collins and Aramaki (1980) reported the inhibition of *Staphylococcus aureus* and *Pseudomonas fragi* by certain LAB strain which is as a result of the antimicrobial substances they possess which have inhibitory activity against other micro organisms, including food borne pathogens (Whittenbury, 1964; Dahiya and Speck, 1968; Price and Lee, 1970; Gilliand et al., 1984).

The highest yield of phytic acid was produced by *L. plantarum* and this can be attributed to its ability to break down the substrate (maize) to release the phytic content of the cereal (Maga, 1981).

Most of the LAB isolates studied produced protease enzyme as opposed to their non ability to produce cellulase. The ability to produce more of protease is as a result of the LAB to break down protein more than carbohydrate and thus this will require the activity of the protease enzyme.

All LAB isolates survived freezing and spray drying conditions. *L. plantarum* and *P. acidilactici* grew very well during freezing. LAB isolates had a lower survival rate when exposed to spray drying as opposed to freezing conditions and this might be attributed to the thermal and dehydration inactivation simultaneously experienced by the cells during spray drying (Brian and Etzel, 1997).

Conclusion

It can therefore be concluded that even among the same species of LAB isolates obtained from fermented maize samples from the same state or different state, there was a marked difference in their physiological characters, thus if they are to be used as starter cultures in the fermentation of maize to produce masa, those with favourable physiological properties like *L. fermentum, L. plantarum and P. acidilctici* could be used. These organisms have proved to be favourable in terms of withstanding a low pH (a very acidic environment), freezing and spray drying conditions, excessively high and low temperatures, reduced ability to produce phytate, production of high concentration of lactic acid and hydrogen peroxide, and ability to produce protease and amylase enzyme. Thus, this will prove to be a good starter culture to be used for the fermentation of maize to give rise to an improved masa production.

The phenotypic diversity and composition of the LAB flora varied as a function of the sampling location, the quality of the maize, initial flora of the maize grain and the length of time of fermentation.

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