Global Scientific JOURNALS

GSJ: Volume 7, Issue 12, December 2019, Online: ISSN 2320-9186

www.globalscientificjournal.com

PHENOTYPIC CHARACTERIZATION AND TECHNOLOGICAL PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTING MAIZE

Oluseun I. Ogundeji, Bridget O. Omafuvbe, Taiwo O. Ogunwale
Department of Microbiology
Obafemi Awolowo University, Osun State, Nigeria
Email-seunogundeji@yahoo.co.uk

Abstract

Twenty-five strains of lactic acid bacteria (LAB) were isolated from fermenting maize. The phenotypic and technological properties were investigated with the aim of selecting potential starter cultures for controlled fermentation. Changes in LAB counts, pH, titratable acidity were observed at 24 hourly interval for 72 h during the natural fermentation of maize. Technological properties such as amylase production, acidification and exopolysaccharide production by the isolates were also investigated. Results revealed that LAB count increased from 6.90±0.05 to 7.61±0.01 log cfu/ml from 0 h -72 h fermentation time. The isolates were identified as *Lactobacillus fermentum* (8%), *L. plantarum* (64%), *L. brevis* (20%), *L. delbrueckii* (4%) and *Streptococcus lactis* (4%). pH decreased from 6.56±0.06 to 5.90±0.44 while the titratable acidity increased from 0.11±0.03 to 0.16±0.05 mg lactic acid/g in the fermenting maize grains from zero to the 72nd hour of natural fermentation respectively. The technological studies on the LAB strains showed that none of them exhibited amylase activity, eleven isolates established rapid acidifying activity while only one showed slow acidifying activity. Twelve isolates were good producers of exopolysaccharides while five of the isolates produced EPS poorly. This work however demonstrated that lactic acid bacteria involved in the natural fermentation of maize can be used as starter culture for fermentation of maize-based products.

Keywords:

Fermentation, lactic acid bacteria, Maize, starter culture, Technological properties, Amylase activity, Acidifying activity

Introduction

Fermentation is an old technology which is reliant on the biological act of microorganisms for the production of diverse metabolites that can control the growth and continued existence of unnecessary microflora in food (Klaenhammer and Fitzgerald, 1994). The fermentation techniques are frequently on small scale and household basis, characterized by the use of necessary non-sterile equipments, chance inoculums, unregulated environment, sensory fluctuations, poor stability and unlikeable packing of the processed produce resulting in food of irregular quality (Olanrewaju *et al*., 2009). Fermentation is a way of improving the sensory qualities and satisfactoriness of raw materials to such a level that numerous foods are preferred in their fermented condition than in the raw material.

Cereal grains consist of a fundamental group of substrate for fermented foods. It is considered to be one of the key sources of dietary proteins, carbohydrates, vitamins, minerals and fibre for people all over the world (Blandino *et al.,* 2003).

Maize is a cereal grain which can be used in the production of traditional fermented foods that are well known in most part of the world. Some are utilized as colorants, spices, beverages and breakfast or light meal foods while a few of them are used as main foods in the diet (Blandino *et al.,* 2003).

A wide range of microorganisms have been implicated during the fermentation process, but a small amount of them determines the quality of the food product (Olaoluwa *et al*., 2013). Lactic acid bacteria are central in the fermentation of lots of traditional foods and beverages. They are found in different stages of fermentation processes where they are helpful in flavour and aroma improvement. They also restrain spoilage bacteria and pathogens and bestow several health benefits (Olaoluwa *et al*., 2013). They can also be used as starter culture to advance the sensory and nutritional properties of foods (Holzapfel *et al.*, 1995). Although maize is transformed into finished food products, inconsistency usually sets in ranging from high to low quality products from batch to batch which could be as a result of the combined activities of the LAB strains, hence the need to isolate and characterize lactic acid bacteria associated with the natural fermentation of maize as well as identify and select the best lactic acid bacteria candidates based on their technological properties as starter culture for consistent quality of maize-based products.

Materials and Methods

Sample Collection

Maize grains used in this study were purchased at a retail market in Ile-Ife, Osun State, Nigeria. It was transported in a polythene bag to the laboratory in the Department of Microbiology, Obafemi Awolowo University, Ile-Ife and stored at room temperature in a dry place.

Traditional Fermentation of Maize Grains

The traditional method of fermentation described by Teniola and Odunfa (2002) was used. The grains (500 g) were washed and steeped in tap water (1.5 L) in triplicates in conical flasks. The conical flasks were covered with aluminium foil and left to ferment for 72 h at room temperature (30°C±2°C). Triplicate samples were collected for analysis at 24 h intervals during the fermentation period.

pH Determination

The pH of the samples collected at regular interval during fermentation was determined according to the method of AOAC (2000) using a pH meter (Hanna instrument 8021). The fermenting maize grain was homogenized and diluted 10 folds and the pH of the resulting solution was measured. The pH of the fermenting maize liquor (10 ml) was also determined.

Determination of Titratable Acidity

The total titratable acidity in the fermenting samples was determined by titration against 0.1 N NaOH, using phenolphthalein (1% w/v) as indicator. Briefly, to 20 ml of the fermenting maize liquor, was added 3 drops of phenolphthalein and titrated with 0.1N NaOH slowly until a pH of 8.30 (which is the end point) was attained. Fermenting maize liquor collected at the different time intervals was titrated in triplicate. Also, 3 drops of phenolphthalein were added to 20 ml filtrate of the homogenized fermenting grain samples (10 g of maize grains in 90 ml of distilled water) and titrated with 0.1 N NaOH until the end point was reached. Each ml of 0.1 M NaOH is equivalent to 90.08 mg of lactic acid (A.O.A.C., 2000).

$$mg\ Lactic\ acid = \frac{N \times V \times ME\ of\ lactic\ acid}{Volume\ of\ sample}$$

Where N is the normality of the sodium hydroxide

V is the sodium hydroxide in ml used to reach the end point

ME is the mill equivalent of lactic acid = 90.08 mg

Isolation and Identification of LAB

Ten milliliter of sample were homogenized with 90 ml of maximum recovery diluent (MRD, Oxiod) and serially diluted in the same diluent. 0.1 ml of appropriately diluted sample was spread-plated in MRS agar (de Man, Rogosa, and Sharpe agar). Plates were then incubated in anaerobic jar provided with disposable BBL gas generating pack (CO_2 system envelopes, Oxoid) at 30°C for 48h and the viable LAB counts were determined. Representative strains of LAB were obtained and purified by successive subculturing on MRS agar. Characteristics of the isolated strains such as catalase test, Gram staining, morphology, and motility test were studied. Strains which were catalase negative and Gram positive were preliminary identified as LAB.

Further identification was performed by using the following tests: growth at different temperatures (15 and 45°C), different pH (3.9 and 9.6) as well as the ability to grow in different concentrations of NaCl (4.0 and 6.5%) in MRS broth; gas production from glucose, determined in MRS broth containing inverted Durham tube; hydrolysis of arginine tested on MRS – Arginine broth, citrate utilization; and production of acetoin from glucose, determined by using the Voges-Proskauer test. The fermentation of carbohydrates was done in modified MRS broth (in which meat extract and glucose were omitted) containing phenol red (0.04 gL^{-1}) as a pH indicator, and supplemented with 1% of the following carbohydrates; xylose, galactose, sorbitol, mannitol,

maltose, melibiose, ribose, trehalose, salicin, lactose, raffinose, cellobiose, sucrose and mannose (Omafuvbe and Enyioha, 2011).

.

Technological Properties of LAB

Acidification Activity

The acidification activity of the LAB isolates was determined following the method described by Kostinek *et al.* (2005). MRS broth medium for all acid production tests was prepared from a single batch which was adjusted to pH 6.5, dispensed into tubes and autoclaved at 121°C for 15 mins. The sterile broth was then inoculated with 18 h old broth culture of each strain at 1% level and incubated at 30°C. Acid production was determined by measuring the pH of the culture after 6, 24, and 48 h. Acidification was calculated using the equation below:

ΔpH= pH at time **–** pH zero time.

where ΔpH= rate of acidification.

The cultures were considered as fast acidifying when a ΔpH of ≥ 1.0 U was achieved at 6 h, medium acidifying when a ΔpH of 0.6 – 0.99 U was achieved and slow acidifying when a ΔpH of < 0.6 U was achieved.

Assessment of Amylolytic Activity

Surface dried plates of starch agar were streaked with 24 h old culture of LAB and incubated at 30°C for 4 days. The plates were flooded with Gram`s iodine solution for 15 to 30 min and examined for clear zones around and underneath the growth for assessment of amylolytic activity (Omafuvbe and Enyioha, 2011).

Exopolysaccharide Production

The test for EPS production by the isolates was carried out following the method described by Guiraud (1998). The lactic acid bacteria strains precultivated on MRS agar was streaked on LTV agar (0.5% (w/v) tryptone, 1% (w/v) meat extract, 0.65% (w/v) NaCl, 0.8% (w/v) KNO_3, 0.8% (w/v) Sucrose, 0.1% (v/v) Tween 80, 1.7% (w/v) agar), pH 7.1± 0.2) and incubated at 30°C for 48 h (Sawadogo-Lingani *et al.,* 2007). The sticky aspect of the colonies was determined by testing them for slime formation using the inoculating loop method (Knoshaug *et al.,* 2000). Positive results were confirmed using MRS –Sucrose broth without glucose and peptone as previously described (Omafuvbe and Enyioha, 2011).

Statistical Analysis

The various data obtained in this study were subjected to analysis of variance and the Student - Newman - Keuls test. It was used to determine significant difference between means using primer for Biostatistics version 3.01 software (Glantz, 1992). Statistical significance was accepted at P value equal to or less than 0.05 ($P \leq 0.05$).

Results and Discussion

The changes in pH of the maize during the natural fermentation is shown in Table 1. The pH of the maize grains decreased from 6.56±0.06 to 5.90±0.44 while the pH of the steep liquor decreased rapidly from 6.50 to 4.17 at the 72nd hour of fermentation. The decrease in pH could be as a result of accelerated growth rate and metabolic activities of LAB responsible for fermentation (Inyang and Idoko, 2006).

The Total Titratable Acidity of the fermenting maize increased slightly from 0.11±0.03 to 0.16±0.05 mg lactic acid/g while that of the steep liquor increased quite rapidly from 0.11±0.03 to 1.55±0.28 from zero hour to the 72nd hour (Table 2). The high levels of TTA recorded in the traditional fermented samples may be attributed to the high acid production as indicated by the reduction in pH. The cause of the increase in acidity and consequent drop in pH during fermentation of cereal was likely due to utilization of free sugars by yeasts and LAB (Omemu, 2011).

Table 3 shows the viable counts of LAB from the maize steep water which increased from zero hour till the 48th hour of fermentation and decreased at the 72nd hour. The reduction in the number of lactic acid bacteria (LAB) isolates may be due to their varying sensitivity to acidity (Adams, 1995).

A total of twenty five lactic acid bacteria strains were isolated from the fermenting maize liquor. The characteristics exhibited by the isolates were compared with those of standard strains for their identification (Harrigan and McCance, 1998).The isolates were all Gram positive, rods and cocci. They were negative to catalase and nitrate reduction and were biochemically characterized as *L. fermentum, L.plantarum, L.brevis, L.delbrueckii and S. lactis.*

Table 4 shows the occurrence pattern of lactic acid bacteria strains during the traditional fermentation of maize. *L. fermentum* occurred mostly at the initial stages of the fermentation while *L. plantarum* with *L. brevis* appeared at the later stages. *L. delbrueckii* and *S. lactis* also occurred at the 48th and 72nd hour of fermentation respectively. The most frequently isolated LAB species during the fermentation were *L. plantarum* followed by *L. brevis*. The frequencies of dominance of the lactic acid bacteria strains associated with the natural fermentation were *L. plantarum* 64%, *L. brevis* 20%, *L. fermentum* 8%, *L. delbrueckii* 4% and *S. lactis* 4% as shown in Figure 1. As in the earlier reports on the occurrence of lactic acid bacteria spectrum, *L. plantarum* constituted the highest number of LAB isolated from some Nigerian fermented foods such as *ogi, nunu, fufu*, etc. The involvement of various types of LAB in fermented vegetables and plant materials had earlier been reported (Adebayo-tayo and Onilude, 2008; Sutherland, 1994).

Technological properties of predominant LAB strains from fermented maize grains

Amylase activity

None of the predominant LAB strains isolated during the fermentation of maize showed amylase activity. This agrees with the findings of other investigators (Vinodh *et al*., 2012) that could not detect amylase producing lactic acid bacteria during the processing of dolo and pito (cereal based fermented product). Although, this rare trait among LAB strains isolated from fermenting starchy-based foods product seems surprising, however, amylolytic lactic acid bacteria have been reportedly isolated from starchy fermented foods in Africa such as fermenting cassava and maize (Sanni *et al.,* 2002).

Acidification Activity

Figure 2 shows the acidification activities of *L. fermentum, L. delbruckii* and *S. lactis* isolated from natural fermentation of maize. *L. delbrueckii* had the lowest acidification activity (having acidification rate of 0.6 U, 0.65 U and 0.5 U at the 6th, 24th and 48th h respectively) compared to *L. fermentum* and *S. lactis* strains. *S. lactis* had the highest acidification activity throughout the fermentation period when compared to the other strains (1.05 U, 1.2 U and 1.15 U). Figure 3 shows the acidification activity of the different strains of *L. brevis.* Of these, *L. brevis* (I_2) had the lowest acidification activity with an acidification rate of 0.6 U and 0.65 U at the 24th and 72nd hour respectively, while *L. brevis* (H_4) had the highest acidification activity from the 6th hour to the 24th hour. All the strains had their peak rate of acidification at the 24th hour of fermentation.

The acidification activities of *L. plantarum* strains are shown in Figure 4. *L. plantarum* (G_2) had the lowest acidification rate of 0.55 U at 6 h, 0.6 U at 24 h and 0.45 U at 48 h. *L. plantarum* (M_4) had the highest acidification rate of 1.2 U at 6 h which was stable till the 24th hour and increased at the 48th hour. The cultures were classified as fast acidifying when a ΔpH of ≥1.0 U was achieved, medium acidifying when a ΔpH of 0.6-0.99 U was achieved and slow acidifying when a ΔpH of <0.6 U was achieved at 6 h. Acid production ability is important because it enhances the biosafety of food products. The potential starter strains which acidify their environment quickly is known to extend the lag phase of food borne pathogens (Mathara *et al.,* 2008).

Exopolysaccharide (EPSs) Production

The ability of lactic acid bacteria isolated from fermenting maize to produce EPS is shown in Table 5. Of the 25 isolates screened, (68%) produced slime. However, EPS confirmation test showed that 48% were good producers of EPS (3+) while 20% produced EPS poorly (2+). This is however not a surprise since many strains of LAB have been reported to produce exopolysaccharide (Geel-Schutten *et al.,* 1998).

Conclusion

In conclusion, lactic acid bacteria were characterized by biochemical methods before subjecting them to technological characterization. Results however revealed that *Lactobacillus plantarum* (G_4), *Lactobacillus plantarum* (M_4) and *Streptococcus lactis* (A_4) could be selected as a potential starter culture. It is however recommended that further studies should be carried out on the molecular characterization of the selected strains and their use on specific maize based products should be investigated.

Acknowledgement

Microbiology Technical Staff, Microbiology Department, Obafemi Awolowo University, Ile-Ife, Nigeria is appreciated for releasing most of the materials needed for this work. I also appreciate the faculty of science and pharmacy for their input during the period of the research which brought about its successful completion

References

1. Adams, M. R. (1995). Topical aspects of fermented foods. *Trends in Food Science and Technology,* 1: 141-144.

2. Adebayo-Tayo, B. C. and Onilude, A. A. (2008). Screening of lactic acid bacteria strains isolated from some Nigerian fermented foods for EPS production. *World Applied Sciences Journal,* 4(5): 741-747.

3. A.O.A.C. (2000). *Official Methods of Analysis. 17th Edition*. Association of Official Analytical Chemists, Gaithersburg, U. S. A.

4. Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D and Webb, C. (2003). Cereal-based fermented foods and beverages. *Food Research International*, 36(6): 527-543.

5. Geel-Schutten, G. H., Flesch, F., Ten Brink, B., Smith, M.R. and Dijkhuizen, L. (1998). ''Screening and characterization of lactobacillus strains producing large amounts of exopolysaccharides'' *Applied Microbiology and Biotechnol*ogy, 50: 697-703.

6. Glantz, S. A. (1992). *Primer of Biostatistics*: The Program. McGraw-Hill Inc.

7. Guiraud, E., Champailler, A., Moulard S. and Raimbault, M. (1998). ''Development of a miniaturized selective counting strategy of lactic acid bacteria for evaluation of mixed starter culture in a model cassava fermentation'', *Journal of Applied Microbiology*, vol. 84, pp. 444-450.

8. Harrigan, W. F. and McCance, M. E. (1998). Biochemical test for bacteria. In Harrigan, W. F. and McCance, M.E. (Eds.), *Laboratory Methods in Food and Dairy Microbiology*. Academic Press. London, New York. pp. 66-71.

9. Holzapfel, W. H., Geisen, R. and Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology*, 24: 343–362.

10. Inyang, C.U. and Idoko, C.A. (2006) Assessment of the quality of *ogi* made from malted millet. *African Journal of Biotechnology*, 5(22):2334-2337.

11. Kleanhammer, T. R. and Fitzgerald, G. F. (1994). Bacteriophage and bacteriophage resistance. In: Gasson M.J., de Vos W.M. Eds. *Genetics and Biotechnology of Lactic Acid Bacteria.* Chapman and Hall London,Englang. p 106 – 168.

12. Knoshaug, E. P., Ahlgrent, J. A. and Trempy J. E. (2000). ''Growth associated polysaccharide expression in *Lactococcus latis* sub spp *cermoris*'', *Journal of Dairy Science*, vol. 83, pp. 633 – 640.

13. Kostinek, M., Ingrid, S., Vinodh, E., Ulrich, S., Christian, H., Wilhelm, H. and Charles, M. A. P. (2005). Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of garri, a traditional African food. *Systematic and Applied Microbiology*, 28: 527 – 540.

14. Manthara, J. M., Schillinger, U., Guigas, C., Franz, C., Kutima, P. M., Mbugua, S. K, Shin, H. K and Holzapfel W. H. (2008). Functional characteristics of lactobacillus spp from traditional Maasai fermented milk products in Kenya. *International Journal of Food Microbiology*, 126 (1-2): 57-64.

15. Olanrewaju, O. O., Victor, O. O. and Titilayo, T. A. (2009). Safety of small-scale food fermentations in developing countries. *Internet Journal of Food Safety*, 11: 29-34.

16. Omafuvbe, B. O. and Enyioha, L. C (2011). Phenotypic identification and technological properties of LAB isolated from selected commercial Nigerian bottled yoghurt. *African Journal of Food Science*, 5(6): 340-348.

17. Omemu, A. M. and Faniran, O. W. (2011). Assessment of the antimicrobial activity of lactic acid bacteria isolated from two fermented maize

products – ogi and kunnu-zaki. *Malaysian Journal of Microbiology* **7(3):** 124–128.

18. Olaoluwa, O., Ogunbanwo, S. T. and Onilude, A. A. (2013). Predominant lactic acid bacteria involved in the traditional fermentation of fufu and *ogi*, two Nigeria fermented food products. *Scientific Research, Food and Nutritional Sciences,* 4: 40-46.

19. Sanni, A., Morlon-Guyot, J. and Guyot, J.P. (2002). New efficient amylase-producing strains of *Lactobacillus plantarum* and *L. fermentum* isolated from different Nigerian traditional fermented foods. *International Journal of Food Microbiology*, 72:53–62.

20. Sawadogo-Lingani, H. Lei, V., Diawara, B., Nielsen, D. S., Moller, P. L., Traore A. S. and Jakobsen, M. M. M. (2007). ''The biodiversity of predominant lactic acid bacteria in dolo and pito wort for the production of Sorghum beer''. *Journal of Applied Microbiology*, vol. 103, no. 4, pp. 765-777.

21. Sutherland, I. W. (1994). Structure-function relationships in microbial exopolysaccharides. *Biotechnology Advanced*, 12: 393-448.

22. Teniola, O. D. and Odunfa, S. A. (2002). Microbial assessment and quality evaluation of *ogi* during spoilage. *World Journal of Microbiology and Biotechnology*, 18, 731-737

23. Vinodh, A. E., Moutairou, E., Melanie, H., Petrus, J. V., Suren, S., Naledzani, D. N., Vetja, M. H. and Charles, M. A. P. F. (2012). Isolation and screening of microorganisms from a gari fermentation process for starter culture development. *Africal Journal of Biotechnology*, 11(65): 12865-12877.

Table 1: Changes in pH during the Natural Fermentation of Maize

	pH	
Fermentation time (h)	Grains	Liquor
0	6.56 ± 0.06^{af}	6.50 ± 0.00^{af}
24	6.47 ± 0.15^{ag}	4.70 ± 0.17^{dh}
48	6.40 ± 0.10^{ag}	4.60 ± 0.50^{cdh}
72	5.90 ± 0.44^{bh}	4.40 ± 0.17^{edi}

Values are mean ± SD where n=3. Mean with different superscript in the same column or row are significantly different (P<0.05).

Table 2: Changes in Total Titratable Acidity during the Natural Fermentation of Maize

	TTA (mg lactic acid)	
Fermentation Time (h)	Grains	Liquor
0	0.11 ± 0.03^{ac}	0.11 ± 0.03^{bc}
24	0.11 ± 0.03^{ad}	0.45 ± 0.12^{abe}
48	0.12 ± 0.03^{ad}	1.07 ± 0.21^{abe}
72	0.16 ± 0.05^{af}	1.55 ± 0.28^{af}

Values are mean ± SD, where n=3. Mean with different superscript within the same column or row are significantly different (P<0.05).
TTA is expressed as mg lactic acid/g for maize grains
TTA is expressed as mg lactic acid/ml for the steep liquor

Table 3: Viable Count of Lactic Acid Bacteria during Natural Fermentation of Maize

Fermentation Time (h)	Liquor (log Cfu/ml)
0 h	6.90±0.05a
24 h	7.56±0.02c
48 h	7.85±0.01b
72 h	7.61±0.01dc

Values are mean ± SD, where n=3. Mean with different superscript within the same column or row are significantly different (P<0.05).

Table 4: Occurrence Pattern of LAB during the Natural Fermentation of Maize

	Strains of LAB				
Fermentation Time (h)	*L. fermentum*	*L. plantarum*	*L.brevis*	*L. delbrueckii*	*S. lactis*
0	+	-	-	-	-
24	-	+	+	-	-
48	-	+	+	+	-
72	-	+	+	-	+

Key: - : Absent
+ : Present

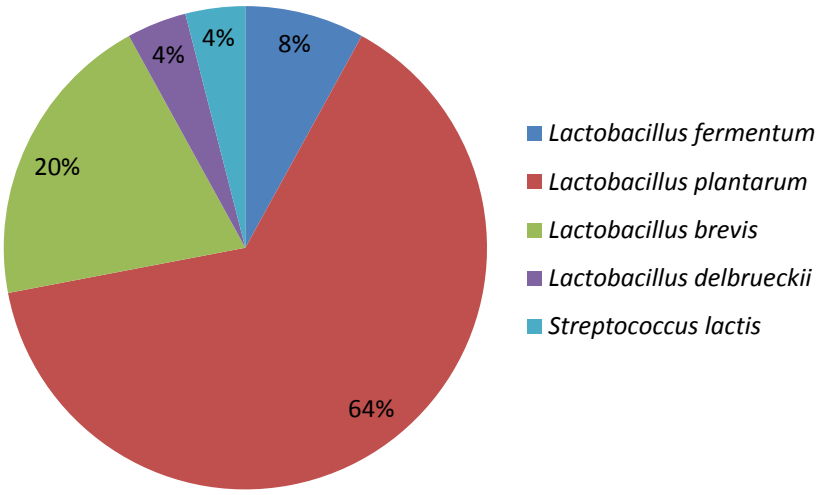

Figure 1: Percentage occurrence of lactic acid bacteria in maize fermentation

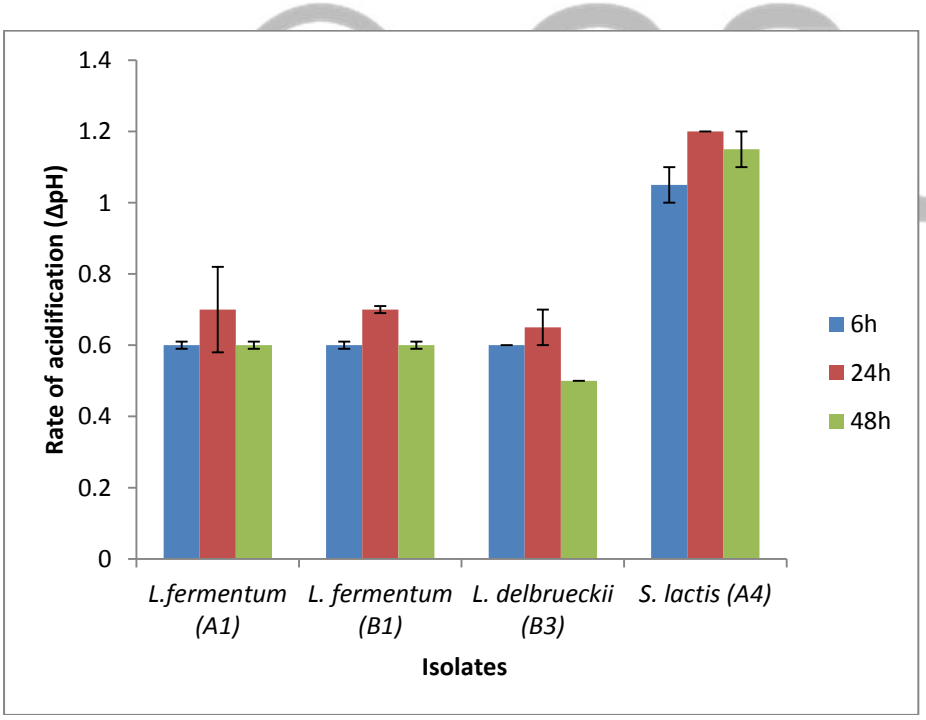

Figure 2: Acidification Activities of *L. fermentum*, *L. delbruckii* and *S. lactis* Strains Isolated from Natural Fermentating Maize

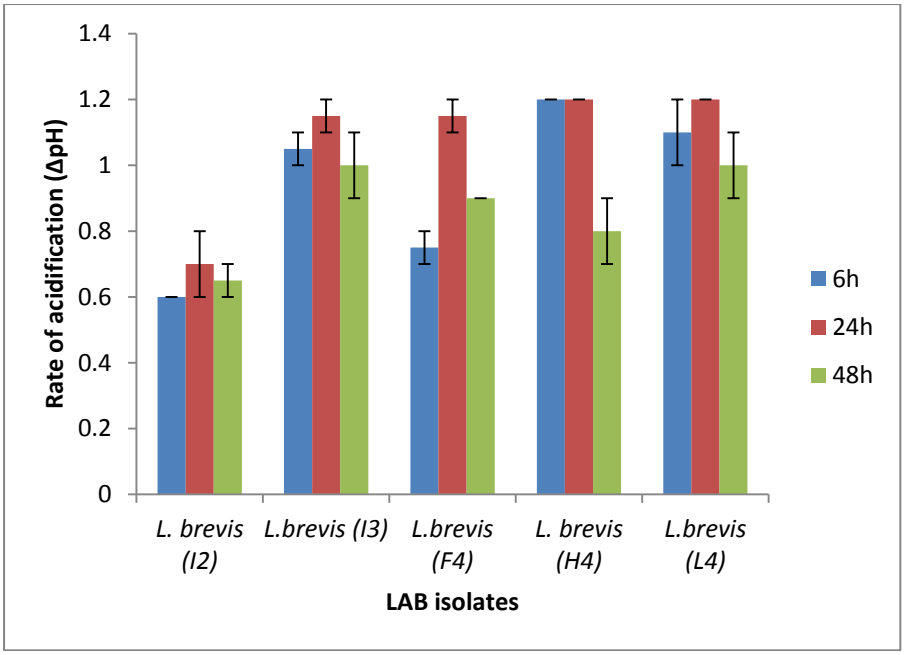

Figure 3: Acidification Activities of *L. brevis* Strains Isolated from Natural Fermenting Maize

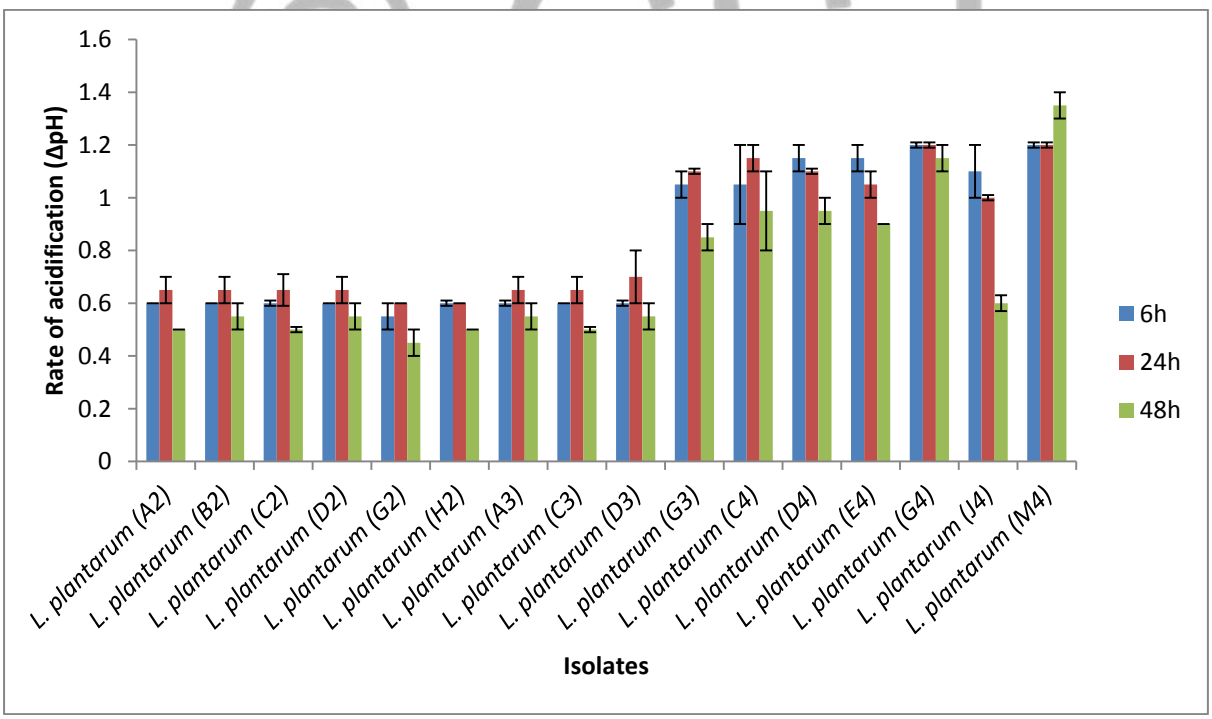

Figure 4: Acidification Activities of *L. plantarum* Strains Isolated from Natural Fermenting Maize

Table 5: Morphological and Biochemical Characterization of Isolates

Sample Source	Maize Grains					
Number of Strains	2	16	4	1	1	1
Gram reaction /Morphology	GPR	GPR	GPR	GPR	GPC	GPR
Catalase	-	-	-	-	-	-
MR	-	-	-	-	-	-
VP	-	-	-	-	+	+
Arginine	+	-	+	-	-	+
MRS_{gas}	+	-	+	-	-	+
pH 3.9	-	+	-	-	+	-
pH 9.6	+	+	+	+	+	+
NaCl 4.0%	+	+	+	+	+	+
NaCl 6.5%	+	+	+	+	+	+
15°C	-	+	+	-	+	+
45°C	+	+	-	+	+	-
Xylose	+	-	-	-	-	+
Galactose	+	+	+	+	+	+

Sorbitol	+	+	+	-	+	+
Mannitol	-	+	+	-	+	+
Maltose	-	+	+	-	+	+
Melibiose	+	+	-	-	+	-
Ribose	+	+	+	-	-	-
Trehalose	+	+	-	-	+	+
Salicin	+	+	-	-	+	+
Lactose	+	+	+	-	+	-
Raffinose	+	+	+	-	+	+
Cellobiose	+	+	-	-	+	+
Sucrose	+	+	-	-	+	+
Mannose	+	+	-	-	+	+
Identification	*L. fermentum*	*L. plantarum*	*L. brevis*	*L. delbrueckii*	*S. lactis*	*L. casei*

Key: GPR – Gram positive rod GPC – Gram positive cocci

L – Lactobacillus S – Streptococcus

- = Negative + = Positive