Original Research Article

Probiotic potentials of lactic acid bacteria isolated from fermented foods

Abstract

This study investigated the probiotic potentials of seven lactic acid bacteria (LAB) strains at different temperatures, pH, and bile salt concentrations. Their antimicrobial activity and antibiotic susceptibility were also determined. There were significant ($P<0.05$) differences in the LAB growth at 45-65^{0}C with viable counts ranging from 4.28-8.34 Log_{10} Cfu/ml after 48 h. The LAB strains showed significant ($P<0.05$) increase at pH 2, 2.5 and 3 after 3 and 6 h. *L. parabuchneri LMG* was viable at 45 and 65^{o}C with 99.30 and 65.00% survival respectively. The LAB showed high resistance to 0.3% bile salt at 97.90%. *L. plantarum CIP* was viable with 95.40% survival at pH 3.0 after 3 h. All the LAB strains were susceptible to cefuroxime (20 µg/ml) and erythromycin (10 µg/ml) at 13.00-45.00 mm zone of inhibition (ZOI). They had strong antimicrobial activity against *Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027* and *Listeria monocytogenes ATCC 15313*. *Leuconostoc mesenteroides LM* and *L. brevis ATCC* inhibited the five tested food borne pathogens with ZOI varying from 8.00-26.00 mm. The results from this study showed that the LAB strains isolated from fermented foods had probiotic potential and can be used for research and commercial purposes.

Keywords: Probiotics, fermented foods, LAB, susceptibility, antimicrobial activity

1.0 Introduction

Probiotics are live strains of carefully selected microorganisms which when consumed in the adequate proportion can improve the intestinal microbiota and promote human health (Sathyabama *et al*., 2014; Aslam and Qazi 2010). *Lactobacillus* and *Bifidobacterium* species are mainly used as food probiotics (Barber *et al*., 2021; Obinna-Echem, 2018). They are believed to be desirable members of the intestinal microflora and have the "Generally Recognized As Safe" (GRAS) status (Ayichew *et al*., 2017; Didari *et al*., 2014). Lactobacilli are present in different food sources such as, cereal-based foods, dairy foods, and fermented foods and beverages (Wejinya et al., 2022; Obinna-Echem *et al*., 2014). Examples of *Lactobacillus* species include, *L. acidophilus, L. paracasei, L. rhamnosus, L. parabuchneri, L. brevis, L. johnsonii, L. plantarum,* and *L. fermentum*. Lactobacilli

exhibit important probiotic properties, including tolerance to high temperature, acid and bile, ability to adhere to intestinal surfaces, strong antimicrobial activity and antibiotics susceptibility, and cholesterol-reducing ability (Tulumoglu *et al*., 2013; Lee *et al*., 2013; Ruiz *et al*., 2013). Several authors have demonstrated the therapeutic evidence of probiotics in prevention and treatment of health problems. These include, alleviation of lactose intolerance, prevention and treatment of diarrhoea, treatment of functional constipation in adults, immune system stimulation, treatment of bacterial vaginosis, lowering of plasma cholesterol, reduction of viral-associated pulmonary damage, and prevention of urogenital diseases (Zelaya *et al*., 2014; Lee *et al*., 2013; Savard *et al*., 2011; Parmjit, 2011; Carlos *et al*., 2010). Recent studies have shown that some strains of lactic acid bacteria (LAB) isolated from fermented foods display attributes desirable for probiotic cultures (Mokoena *et al*., 2016). In Nigeria, probiotics have been isolated from different fermented foods (Wejinya *et al*., 2022; Ngene *et al*., 2019; David *et al*., 2019; Berebon *et al*., 2018; Olokun *et al*., 2018; Obinna-Echem *et al*., 2014). However, the criteria for LAB strains to be characterised as probiotics either for food or nutraceutical applications are constantly evolving and developing. The guidelines proposed by FAO/WHO (2002) for evaluation of probiotics recommended that potential probiotic strains should be well investigated to determine their ability to survive the gastrointestinal tract (GIT), antibiotic susceptibility and antimicrobial activity. Therefore, this study was aimed at investigating the probiotic potentials of lactic acid bacteria isolated from fermented foods.

2.0 Materials and Methods

2.1 Lactic acid bacterial strains and inoculum preparation

The seven potential probiotic LAB strains characterised in this study were previously isolated from ogi, fufu, nunu, palmwine and fermented tigernut milk (Wejinya *et al*., 2022). The potential probiotic LAB strains were identified using both API 50 CHL (Biomerieux, France) and molecular

techniques. These LABS are: *Lactobacillus fermentum NBRC 15885* (ogi), *Leuconostoc mesenteroides LM* (ogi), *Lactobacillus plantarum CIP 10315.1* (fufu), *Lactobacillus plantarum NBRC 15891* (tigernut), *Lactobacillus parabuchneri LMG 11457* (tigernut), *Lactobacillus pentosus 124-3* (palmwine) and *Lactobacillus brevis ATCC 14869* (nunu).

The inoculum was prepared using the method described by Obinna-Echem (2018) with slight modifications. The LAB strains were inoculated from slants to 10 mL of fresh MRS broth containing 1% glucose and incubated at 45°C for 16 - 18 h. The cultures were harvested by centrifugation at 5000 rpm for 20 min at 4°C and washed twice in phosphate buffered saline (PBS) (pH 7.2). The cells were re-suspended in PBS such that 1 mL of inoculum produced 9 Log_{10} Cfu/mL. The media and the diluent used were obtained from Oxoid Limited (Basingstoke, Hampshire, UK).

2.2 Growth at different temperatures

The growth of the LAB strains at different temperatures were studied using the method described by Mulaw *et al*., (2019). A volume of 1 ml of each washed cells were diluted in sterile 9 ml sterile MRS broth and incubated at 45, 55 and 65°C for 48 h under anaerobic condition using an anaerobic jar (BBL, Gas Pack System). Survival were measured by plating out serial dilutions on MRS agar plates at the beginning and end of the incubation time. Percentage survival was calculated as:

$$\text{Survival rate (\%)} = \frac{\text{Viable LAB colonies of each sample at 48 h}}{\text{Viable LAB colonies of each sample at 0 h}} \times 100$$

2.3 Growth at different pH and time

The growth of the LAB isolates were studied at pH 2.0, 2.5, 3.0 using the method described by Grosu-Tudor and Zamfir, (2012). A volume of 1 ml of each washed cells were diluted in sterile 9 ml modified MRS broth which was adjusted to pH values of 2.0, 2.5, and 3.0 using 5 M HCl to simulate

the gastric environment. All the samples were incubated at 45°C for 3 and 6 h under anaerobic condition using an anaerobic jar (BBL, Gas Pack System). After the incubation period, 1 ml of the culture was diluted in sterile 9 ml phosphate buffer (Sigma, St. Louis, MO USA) prepared according to the manufacturer's instruction (0.1 M, pH 6.2) in order to neutralize the medium acidity. Survival was measured by plating out serial dilutions on MRS agar plates at 45°C for 48 h under anaerobic condition using an anaerobic jar (BBL, Gas Pack System). Percentage survival was calculated as:

$$\text{Survival rate (\%)} = \frac{\text{Viable LAB colonies of each sample at 48 h}}{\text{Viable LAB colonies of each sample at 0 h}} \times 100$$

2.4 Tolerance to bile salts

Shokryazdan *et al*., (2014) reported that the normal concentration of bile salt in human small intestine is 0.3% (w/v) hence, this study also used 0.3% bile salt. The staying time of food in small intestine is suggested to be 4 hours (Prasad, *et al*., 1998). The experiment was applied at this concentration of bile for 4 hours. According to the method described by Mulaw *et al*., (2019), one ml of the washed cells were diluted in 10 ml modified MRS broth containing 0.3% oxgall bile salts (Oxoid, UK) and incubated anaerobically at 45^0C for 4 h. Viable colonies were enumerated before the incubation time and after 4 h by plating out serial dilutions on MRS agar plates at 45°C for 48 h under anaerobic condition using an anaerobic jar (BBL, Gas Pack System). Percentage survival was calculated as:

$$\text{Survival rate (\%)} = \frac{\text{Viable LAB colonies of each sample at 48 h}}{\text{Viable LAB colonies of each sample at 0 h}} \times 100$$

2.5 Antimicrobial activity of LAB against food borne pathogens

Antimicrobial activity of LAB strains against some food-borne pathogens was determined using the agar-well diffusion method described by Fontana *et al*., (2013). Pure cultures of *Staphylococcus aureus ATCC 25923, Listeria monocytogenes ATCC 15313, Salmonella enterica typhimurium ATCC 14023, Pseudomonas aeruginosa ATCC 9027, Enterococcus faecalis ATCC 29212* and *Escherichia coli ATCC 25922* were inoculated from slants to Luria Broth (LB). After 24 h incubation at 37°C, 100 µl of the inoculum of each indicator bacteria was spread evenly over the surface of MRS agar plates with a sterile cotton swab. The plates were allowed to dry for an hour. A sterile cork borer of diameter 2 mm was used to cut uniform wells in the agar. The LAB strains were inoculated from slants to fresh MRS broth containing 1% glucose and incubated at 45°C for 16 - 18 h. The supernatant from each culture were obtained as crude extract through centrifugation at 5000 rpm for 20 min at 4°C. Each well was filled with 100 µl of the supernatant obtained from each of the LAB isolates and incubated at 45°C for 24 h under anaerobic condition using an anaerobic jar (BBL, Gas Pack System). The plates were observed for zone of inhibition around the well. Inhibition zones ≤20 mm, >20 mm and ≤10, and ≥9 mm were considered as Susceptible (S), Intermediate (I) and Resistance (R) respectively. The experiment was carried out in triplicates.

2.6 Antibiotic susceptibility test

Each LAB strain was assessed for antibiotic resistance/susceptibility using disc diffusion method as described by Guo *et al*., (2016). The antibiotics used were gentamycin (10 µg/ml), ampiclox (30 µg/ml), cefuroxime (20 µg/ml), amoxacillin (30 µg/ml), ciprofloxacin (10 µg/ml), streptomycin (30 µg/ml), septrin (30 µg/ml), and erythromycin (10 µg/ml). A volume of 100 µl of actively growing LAB culture was spread evenly on the surface of nutrient agar plates using a sterile cotton swab. After drying, the antibiotic discs were placed on the solidified agar surface and allowed to diffuse for 30 min at 4°C. Thereafter, the plates were incubated at 45°C for 24 h under anaerobic conditions using an anaerobic jar (BBL, Gas Pack System).

The zone of inhibition for each antibiotic was measured and expressed as susceptible, S (≥21 mm); intermediate, I (9 - 20 mm), and resistance, R (≤9 mm). The experiment was carried out in triplicates.

2.7 Statistical analysis

All experiments were done in three replicates and data obtained from analysis were statistically analysed using Minitab (Release 18.1) Statistical Software English (Minitab Ltd. Coventry, UK). Statistical differences and relationship among variables were evaluated by analysis of variance (ANOVA) under general linear model and Fisher pairwise comparisons at 95% confidence level.

3.0 Results and Discussions

3.1 Growth at different temperatures

The percentage survival of the LAB strains at 45^0C were 87.5 - 99.3%. The % survival decreased significantly (P>0.05) at 55^0C (63.6 - 75.5%) and 65^0C (51.0 - 65.0%) respectively (figure 1). At 45^0C, *L. parabuchneri LMG*, *L. fermentum NBRC* and *L. brevis ATCC* had % survival of 99.3, 98.3 and 96.4% respectively. Other studies have shown that some thermophilic LAB strains grow well and present highly activated metabolism at around 45^0C (Da Silva *et al*., 2018; Matejˇceková *et al*., 2016; Meena *et al*., 2014). According to FAO/WHO (2002) guidelines, the minimum concentration of probiotics that is required for beneficial effects at the point of consumption should be more than 6 Log_{10} Cfu/ml. All the LAB strains met the minimum concentration for probiotics at 45^0C with viable counts ranging from 7.35 - 8.34 Log_{10} Cfu/ml. Ukwuru and Ohaegbu, (2018) noted that high probiotic cell counts are recommended to allow possible reduction in the population of the organisms during passage through stomach and the intestines.

3.2 Growth at different pH and time

All the LAB strains survived pH 2.0 at 3 and 6 h with % survival of 50.60 - 64.20% and 46.40 - 52.90% respectively as shown in figure 2a. *L. parabuchneri* had the highest survival of 64.2% for 3 h. *L. plantarum NBRC* showed the least survival at 3 and 6 h. Similar to this study, Mourad and Nour-Eddine (2006) have demonstrated that *Lactobacillus spp*. showed % survival of 49.00 - 65.00% when exposed to pH 2.0 for 2 h. Guo *et al*. (2010) reported that the incubation at low pH resulted in significant ($P > 0.05$) decrease in the survival rate of all LAB isolates. However, the result of this study does not agree with the report given by Oh and Jung, (2015) who revealed that 5 acid-tolerant *Lactobacillus* strains showed above 89.00% survival rate after exposure to pH 2 for 3 h. The authors noted that the viable counts of all lactic acid bacteria were significantly affected by low acidity, especially at pH 2.

As shown in figure 2b, the LAB strains were more tolerant and showed significant increase ($P > 0.05$) at pH 2.5 when compared to pH 2.0. The % survival at pH 2.5 ranged from 84.40 - 89.70% for 3 h and 71.40 - 77.70% for 6 h respectively. *Leuconostoc mesenteroides* had the lowest % survival of 84.40% at 3 h and *L. brevis* had the lowest % survival of 71.40% at 6 h. *L. plantarum CIP* had the highest survival at 3 and 6 h with % survival of 89.70% and 77.70% respectively. Similar to these findings, Mulaw *et al*., (2019) reported the survival rate of four *Lactobacillus* strains at pH 2.5 for 3 and 6 h to be tolerant at 71.98 - 97.11% and 65.58 - 90.49%.

The present results were different from those of Mamo *et al*., (2015) who found low to high survival rates 1.03% - 100% for the six *Lactobacillus* species at pH 2.5 and 3.0 for 3 h. The same authors indicated that the maximum survival rate of the six strains were 22.50% at pH 3 for 6 h. This is different from the result obtained in this current study as shown in figure 2c. At pH 3, the % survival was 84.5 - 95.4% for 3 h and 73.3 - 92.3% for 6 h. *L. plantarum CIP* had the highest viability at pH 3 for 3 h with % survival of 95.4% and at 6 h, *L. parabuchneri* showed the highest % survival of

92.3%. Akalu *et al*., (2017) reported a similar result of 81 - 91% at pH 3 for 3 and 6 h. This result also agreed with Azat *et al*., (2016) who reported that the six strains tested were tolerant at pH 3 for 3 h with survival rates ranging from 74.6 - 87.1%. Previous authors have noted that an isolate with full tolerance to pH 3.0 for 3 h can be considered as high-acid-resistant strain with promising probiotic properties (Guo *et al*., 2010; Argyri *et al*., 2013). The potential probiotic LAB strains showed high tolerance at pH 2.5 and 3, exceeding the minimum viable counts of 6 Log_{10} Cfu/ml at 3 and 6 h. This result were similar to the reported presented by Mulaw *et al*., (2019). Moreover, there was significant ($P<0.05$) decrease in the viability of the LAB strains with increase in time which is similar to the result reported by Obinna-Echem, (2018).

3.3 Tolerance to bile salts

There were significant ($P<0.05$) differences in the growth of the LAB strains in 0.3% bile salt for 4 h with % survival of 84.4 - 97.90% as shown in table 1. Similar to the present findings, Haghshenas *et al*., (2017) reported that tested LAB strains displayed high tolerance to bile salt conditions with survival rates of 88 - 92%. In a related study, Akalu *et al*. (2017) showed that 17 out of the 30 tested LAB isolates had high tolerance to an environment containing 0.3% bile salt. The findings of Boke *et al*. (2010) was different from that of the result obtained in this study. The authors reported that *Lactobacillus* strains exhibited low level of tolerance in 0.3% bile salts with survival rates of 36%, 33%, 3%, and 3%, respectively. It is also apparent from the results of the current study that acid tolerance of the LAB strains was not related to the sources of isolation as the level of acid tolerance could vary considerably among the strains from the same source. Oh and Jung, (2015) reported that tolerance to high bile salt condition is strain specific. In this current study, *L. parabuchneri LMG 11457* showed survival of 97.9 % while *L. pentosus 124.3* had the least survival of 84.4%. *L plantarum* strains showed viable counts of 6.27 - 7.38 Log_{10} Cfu/ml in 4 h. This differs from the result of Obinna-Echem, (2018) which showed

that the *L. plantarum* strains tested had viable counts of 5.73 and 7.93 Log_{10} Cfu/ml in 6 h. The ability to tolerate bile salt at a concentration of 0.3% has a physiological significance because it is a level normally encountered in human intestine.

3.4 Antimicrobial activity of LAB against food borne pathogens

The seven potential probiotic LAB strains showed different antagonistic activity against tested pathogens (Table 2). The zones of inhibition varied significantly ($P<0.05$) against the tested food pathogens. All the potential probiotic strains inhibited *Escherichia coli, Pseudomonas aeruginosa,* and *Listeria monocytogenes* with zones of inhibition (ZOI) ranging from 15.00 - 24.00 mm, 19.00 - 25.00 mm, and 21.00 - 26.00 mm respectively. This result is in agreement with the findings of Shokryazdan *et al*., 2014; Srinu *et al*., 2013; Bassyouni *et al*., 2012. Among the seven isolated LAB strains, *Leuconostoc mesenteroides LM* was the most effective strain. It inhibited all the food borne pathogens with clear ZOI of ≥11.00 mm and no resistance. The study also showed that all LAB strains inhibited *Listeria monocytogenes* with high susceptibility of 21.00 - 26.00 mm. *L. plantarum CIP, L. plantarum NBRC* and *L. parabuchneri* had significantly low antimicrobial effects against *Enterococcus faecalis ATCC 29212* with ZOI ranging from 7.00 - 9.00 mm. Similar to the present result, Oluwajoba *et al*., 2013 reported that some LAB strains showed significantly ($P < 0.05$) low antimicrobial effect against *E. faecalis*. The concept of antimicrobial effect of LAB against pathogenic strains has been well documented in a review by Suskovic *et al*. (2010). It is another important attribute to be considered in the selection of potential probiotic strains for maintaining a healthy microbial balance in the GIT. This effect has mostly been attributed to the production of antimicrobial substances or metabolites such as organic acids, ethanol, carbon dioxide, hydrogen peroxide, short-chain fatty acids, and bacteriocins by the probiotic LAB strains (Saulnier *et al*.,

2009). Therefore, by producing these antimicrobial compounds, probiotic microorganisms gain an advantage over other microorganisms to survive in the adverse conditions of the gastrointestinal tract (Handa, 2012).

3.5 Antibiotic susceptibility of the LAB

The antibiotic resistance or susceptibility results (Table 3) showed that all the LAB strains (*L. fermentum NBRC 15885, Leuconostoc mesenteroides LM, L. plantarum CIP 10315.1, L. plantarum NBRC 15891, L. parabuchneri LMG 11457, L. pentosus 124.3 and L. brevis ATCC 14869*) were susceptible to erythromycin and cefuroxime with zones of inhibition (ZOI) ranging from 13.00 - 45.00 mm. *L. plantarum NBRC* 15891 showed the highest susceptibility against cefuroxime with ZOI of 45.00 mm. Three out of these seven LAB strains: *L. plantarum CIP 10315.1, L. pentosus 124.3* and *L. brevis ATCC 14869* were susceptible to all the antibiotics tested with ZOI of 11.00 - 30.00 mm, 13.00 - 26.00 mm and 13.00 - 27.00 mm respectively. Yu *et al*., (2012) reported that the susceptibility of LAB strains may be due to their broad antibacterial spectrum and excellent safety profile. In this current study, *Lactobacillus fermentum NBRC 15885, Leuconostoc mesenteroides LM* and *Lactobacillus plantarum NBRC 15891* showed resistant to gentamycin. Similar results were observed by (Mahantesh *et al*., 2010) who reported that strains of *Lactobacillus fermentum 141* and *Lactobacillus plantarum 20* showed resistant to gentamycin. Naeem *et al*. (2012), tested susceptibility and resistance of 15 isolates against 10 available antibiotics, 50% of all strains were sensitive to the 10 antibiotics used in the test. Sieladie *et al*., (2011), studied fifteen potentially probiotic *Lactobacilli* isolates for antibiotic susceptibility using the agar diffusion method. The LAB strains were sensitive to penicillin, ampicillin, amoxicillin, erythromycin, tetracycline, chloramphenicol, and doxycycline but resistant towards ciprofloxacin. Therefore, it is important to note that each potential probiotic strain has its own specific properties for the antibiotic resistance. Previous reports suggested that resistance of specific

antibiotics promote probiotic applications since probiotics can be administered along with antibiotic therapy and enhance quick recovery of the gut microbiota (Kim and Austin, 2008). Nevertheless, probiotics must be safe for human consumption and should not have transferable antibiotic resistance genes.

4.0 Conclusion and Recommendation

The findings from this work showed that *Lactobacillus fermentum NBRC 15885, Leuconostoc mesenteroides LM, L. plantarum CIP 10315.1, L. plantarum NBRC 15891, L. parabuchneri LMG 11457, L. pentosus 124-3* and *L. brevis ATCC 14869* exhibited probiotic potentials. They survived at temperatures above 45^0C for 48 h. They were viable at pH 3.0 for 3 - 6 h and in 0.3% bile salt for 4 h. All the LAB strains were sensitive to cefuroxime and erythromycin. *L. plantarum NBRC* 15891 showed the highest susceptibility against cefuroxime at 45.00 mm zones of inhibition (ZOI). *L. plantarum CIP 10315.1, L. pentosus 124.3* and *L. brevis ATCC 14869* were susceptible to all the tested antibiotics. *L. plantarum NBRC* showed the highest multi-drug resistance against gentamycin (10 µg/ml), amoxacillin (30 µg/ml), and streptomycin (30 µg/ml). All the LAB strains inhibited *Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027,* and *Listeria monocytogenes ATCC 15313* with ZOI ranging from 15.0 - 26.0 mm. This suggest that these LAB strains isolated from locally fermented maize, cassava, tigernut milk, cow milk and palmwine have good probiotic potentials and can survive passage through the GIT.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Akalu, N., Assefa, F. & Dessalegn, A. (2017). In vitro evaluation of lactic acid bacteria isolated from traditional fermented Shamita and Kocho for their desirable characteristics as probiotics. *African Journal of Biotechnology*, vol. 16, no. 12, pp. 594 - 606.

Argyri, A. A., Zoumpopoulou, G., & Karatzas K. A. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiology, vol. 33, no. 2, pp. 282–291.

Aslam, S. & Qazi, J. I. (2010). Isolation of acidophilic lactic acid bacteria antagonistic to microbial contaminants. *Pakistan Journal Zoology*. 42(5):567 - 573.

Ayichew, T., Belete, A., Alebachew, T., Tsehaye, H., Berhanu, H. & Minwuyelet A (2017). Bacterial Probiotics their Importances and Limitations: A Review. Journal of Nutrition and Health Sciences. 4, Issue 2.

Azat, R., Liu, Y., Li, W., Kayir, A., Lin, D. B., Zhou, W. W. & Zheng, X. D. (2016). Probiotic properties of lactic acid bacteria isolated from traditionally fermented Xinjiang cheese. Journal of Zhejiang University. Science. B, 17(8), 597–609. https://doi.org/10.1631/jzus.B1500250

Barber, L. I., Onuegbu, N. C., Osuji, C. M. & Ogueke, C. C. (2021). Storage Stability of Probiotic Soy Yoghurts with Enzyme Hydrolyzed African Breadfruit and Rice Additives. European Journal of Nutrition & Food Safety. 13(2): 110-121. Article no. EJNFS.65361. ISSN: 2347-5641.

Bassyouni, R. H., Abdel-all, R. H., Fad, M. G., Abdel-all, S. & Kamel, Z. (2012). Characterization of lactic acid bacteria isolated from dairy products in Egypt as a probiotic. *Life Science Journal*, vol. 9, no. 4.

Berebon, D. P., Ofokansi, K. C., Attama, A. A., Eze, C. O., Onwusoba, R. C. & Ugwoke, I. C. (2018). Preliminary studies on isolation, bile tolerance and antibiogram of potential probiotics (Probionts) from locally fermented food products at Beach Market, Nsukka Metropolis, Enugu State, Nigeria. *Biotechnology Journal International*. 22(3): 1-10. Article no.BJI.47409.

Boke, H., Aslim, B. & Alp, G. (2010). The role of resistance to bile salts and acid tolerance of exopolysaccharides (EPSS) produced by yogurt starter bacteria. *Archives of Biological Sciences*, vol. 62, no. 2, pp. 323 - 328.

Carlos, R. S., Luciana, P. V., Michele, R. S., Adriane, B. P., Caroline, T. Y., Juliano, D. L., Ashok, P. & Vanete, T. (2010). The Potential of Probiotics: A Review. *Food Technology and Biotechnology*. 48 (4) 413 - 434.

Da Silva, A. P., Longhi, D. A., Dalcanton, F. & de Aragão, G. M. (2018). Modelling the growth of lactic acid bacteria at different temperatures. *Brazilian Archives of Biology and Technology*. 61, 61.

David, A. A., Orukotan, A. A. & Mohammed S. S. (2019). Conventional and molecular characterization of selected lactic acid bacteria from fermented corn gruel (ogi) and fermented milk (Nono). *Science World Journal*. Vol. 14 (No 4).

Didari, T., Solki, S., Mozaffari, S., Nikfar, S. & Abdollahi, M. (2014). A systematic review of the safety of probiotics. Expert Opinion on Drug Safety. 13, 227 - 239.

FAO/WHO. (2002). Guidelines for the evaluation of probiotics in food; Joint FAO/WHO Working group on drafting guidelines for the evaluation of probiotics in food: London, Ontario, Canada.

Fontana, L., Bermudez-Brito, M., Plaza-Diaz, J., Munoz-Quezada, S. & Gil, A. (2013). Sources, isolation, characterisation and evaluation of probiotics. *British Journal of Nutrition*, vol. 109, no. supplement 2, pp. S35 - S50.

Grosu-Tudor, S. & Zamfir, M. (2012). Probiotic potential of some lactic acid bacteria isolated from Romanian fermented vegetables. *Annals of the Romanian Society for Cell Biology*. 17(1):234 - 239.

Guo, X. H., Kim, J. M., Nam, H. M., Park, S. Y. & Kim, J. M. (2010). Screening lactic acid bacteria from swine origins for multistrain probiotics based on in vitro functional properties. *Anaerobe*, vol. 16, no. 4, pp. 321 - 326.

Guo, X., Chen, D. D., Peng, K. S., Cui, Z. W., Zhang, X. J. & Li, S. (2016). Identification and characterization of *Bacillus subtilis* from grass carp (*Ctenopharynodon idellus*) for use as probiotic additives in aquatic feed. *Fish Shellfish Immunology*. 52 74 - 84. 10.1016/j.fsi.2016.03.017

Haghshenas, B., Nami, Y. & Almasi, A. (2017). Isolation and characterization of probiotics from dairies. *Iranian Journal of Microbiology*, vol. 9, no. 4, pp. 234 - 243.

Handa, S. (2012). Isolation of lactic acid bacteria and to study their potential as probiotics. M.SC Thesis, KrishiKosh, New Delhi, India.

Kim, D. H., & Austin, B. (2008). Characterization of probiotic carnobacteria isolated from rainbow trout (*Oncorhynchus mykiss*) intestine. *Letters in Applied Microbiology*. 47, 141 - 147.

Lee, S. J., Bose, S., Seo, J. G., Chung, W. S., Lim, C. Y. & Kim, H. (2013). The effects of co-administration of probiotics with herbal medicine on obesity, metabolic endotoxemia and dysbiosis: A randomized double-blind controlled clinical trial. *Clinical Nutrition*.

Mahantesh, M. P., Pal, A., Anand, T. & Ramana, K. V. (2010). Isolation and identification of lactic acid bacteria from curd and cucumber. *Indian Journal of Biotechnology*. 9:166.

Mamo, N. J., Assefa, T. F. & Tesfaye, T. A. (2015). Evaluation of the antagonistic effect of six mixed cultures of lactic acid bacteria, isolated from the Ethiopian fermented milk ergo, against some foodborne pathogens inoculated into the Ethiopian cottage cheese ayib. *African Journal of Microbiology Research*, vol. 9, no. 29, pp. 1789 - 1797.

Matejˇceková, Z., Liptáková, D., Spodniaková, S. & Valík, L'. (2016). Characterization of the growth of *Lactobacillus plantarum* in milk in dependence on temperature. *Acta Chimica Slovaca*. 9, 104 - 108.

Meena, G. S., Kumar, N., Majumdar, G. C., Banerjee, R., Meena, P. K. & Yadav, V. (2014). Growth characteristics modeling of *Lactobacillus acidophilus* using RSM and ANN. *Brazilian Archives Biology and Technology*. 57, 15 - 22.

Mokoena, M. P., Mutanda, T. & Olaniran, A. O. (2016). Perspectives on the probiotic potential of lactic acid bacteria from African traditional fermented foods and beverages. *Food and Nutrition Research*. 60: 10.3402/fnr.v60.29630.

Mourad, K. & Nour-Eddine, K. (2006). In vitro preselection criteria for probiotic *Lactobacillus plantarum* strains of fermented olives origin. *International Journal of Probiotics and Prebiotics*, vol. 1, no. 1, p. 27.

Mulaw, G., Tessema, T. S., Muleta, D. & Tesfaye, A. (2019). In Vitro Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products. *Hindawi International Journal of Microbiology*. Volume 2019, Article ID 7179514, 11 pp. https://doi.org/10.1155/2019/7179514.

Naeem, M., Ilyas, M., Haider, S., Baig, S. & Saleem M. (2012). Isolation characterization and identification of lactic acid bacteria from fruit juices and their efficacy against antibiotics. *Pakistan Journal of Botany*. 44, 323 - 328.

Ngene, A. C., Onwuakor, C. E., Aguiyi, J. C., Ifeanyi, V. O., Ohaegbu, C. G., Okwuchukwu, C. P., Kim, E. G. & Egbere, J. O. (2019). Screening of some lactic acid bacteria isolated from selected Nigerian fermented foods for vitamin production. *Advances in Microbiology*, 9, 943 - 955.

Obinna-Echem, P. C. (2018). Acid, Bile and Aggregation Abilities of *Lactobacillus plantarum* Strains Isolated from Akamu a Nigerian Fermented Maize Food. *American Journal of Food Science and Technology*. Vol. 6, No. 1, 7-11. DOI:10.12691/ajfst-6-1-2

Obinna-Echem, P. C., Kuri, V. & Beal, J. (2014). Evaluation of the microbial community, acidity and proximate composition of akamu, a fermented maize food. *Journal of Food Science and Agriculture*. 91:331 - 340.

Oh, Y. J. & Jung, D. S. (2015). Evaluation of probiotic properties of Lactobacillus and Pediococcus strains isolated from omegisool, a traditionally fermented millet alcoholic beverage in Korea. LWT - Food Science and Technology, vol. 63, no. 1, pp. 437 - 444.

Olokun, A. L., Mbagwu, T. T. & Maikori, J. E. (2018). Production of fermented drink from milk extract of tigernut (*Cyperus esculentus*). *South Asian Research Journal of Natural Products*. 1(3): 1-7. Article no. SARJNP.44197.

Oluwajoba, S. O., Akinyosoye, F. A. & Oyetayo, V. O. (2013). Invitro screening and selection of probiotic lactic acid bacteria isolated from spontaneously fermenting kunu-zaki. *Advances in Microbiology*. 3, 309 - 316

Parmjit, S. P. (2011). Fermented dairy products: Starter cultures and potential nutritional benefits. *Food and Nutrition Sciences*. 2:47 - 51.

Prasad, J., Gill, H., Smart, J. & Gopal, P. K. (1998). "Selection and Characterization of Lactobacillus and Bifidobacterium Strains for Use as Probiotic," International Dairy Journal, Vol. 8, No. 12, 1998, pp. 993-1002. doi:10.1016/S0958-6946(99)00024-2

Ruiz, L., Margolles, A. and Sánchez, B. (2013). Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. *Frontiers of Microbiology*. 4, 1 - 8.

Sathyabama, S., Ranjith, K. M., Bruntha, D, P., Vijayabharathi, R. & Brindha, P. V. (2014). Co-encapsulation of probiotics with prebiotics on alginate matrix and its effect on viability in simulated gastric environment. LWT - *Food Science and Technology*. 57:419 - 425. https://doi.org/10.1016/j.lwt.2013.12.024.

Saulnier, D. M., Spinler, J. K., Gibson, G. R. & Versalovic, J. (2009). "Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods," *Current Opinion in Biotechnology*, vol. 20, no. 2, pp. 135–141

Savard, P., Lamarche, B., Paradis, M. E., Thiboutot, H., Laurin, E. & Roy, D. (2011). Impact of *Bifidobacterium animalis subsp. lactis* BB-12 and, *Lactobacillus acidophilus* LA-5-containing yoghurt, on fecal bacterial counts of healthy adults. *International Journal of Food Microbiology*. 149, 50 - 57.

Shokryazdan, P., Sieo, C., Kalavathy, R., Liang, J. B., Alitheen, N. B., Jahromi, M. F. & Ho, Y. W. (2014). Probiotic Potential of *Lactobacillus* Strains with Antimicrobial Activity against Some Human Pathogenic Strains. *BioMed Research International*. Volume 2014, Article ID 927268, 16pp.

Sieladie, D.V., Zambou, N. F., Kaktcham, P.M., Cresci, A. & Fonteh, F. (2011). Probiotic properties of *Lactobacilli* strains isolated from raw cow milk in the western highlands of Cameroon. Innovative Romanian. *Food Biotechnology*. 9(12) 12 - 28.

Srinu, B., Madhava, R. T., Mallikarjuna, R. P. & Kondal, R. K. (2013). Evaluation of different lactic acid bacterial strains for probiotic characteristics. *Veterinary World*. 6(10): 785 - 788.

Suskovic, J., Blazenka, K., Beganovi, J., Pavunc, c, A., Habjani, K. & Matosic, S. (2010). "Antimicrobial activity - the most important property of probiotic and starter lactic acid bacteria," *Food Technology and Biotechnology*, vol. 48, no. 3, pp. 296.

Tulumoglu, S., Yuksekdag, Z. N., Beyatli, Y., Simsek, O., Cinar, B. & Yaşar, E. (2013). Probiotic properties of *Lactobacilli* species isolated from children's feces. *Anaerobe*. 24, 36 - 42.

Ukwuru, M. U. & Ohaegbu, C. G. (2018). Local Cereal Fermented Foods with Probiotic Potentials. *Research Journal of Food and Nutrition*. Volume 2, Issue 1. PP 1 - 13.

Wejinya, A. O., Giami, S. Y., Barber, L. I. & Obinna-Echem, P. C. (2022). Isolation, Identification and Characterization of Potential Probiotics from Fermented Food Products. Asian Food Science Journal, 21(5), 14-25. https://doi.org/10.9734/afsj/2022/v21i530427

Yu, Z., Xue, Z., Sheng-yu, L., Chang-ying, L., Da, L. & Zhen-nai, Y. (2012). In vitro evaluation of probiotic properties of *Lactobacillus plantarum* strains isolated from Chinese Sauerkraut. *African Journal of Biotechnology*. 11: 4868.

Zelaya, H., Tsukida, K., Chiba, E., Marranzino, G., Alvarez, S., Kitazawa, H., Agüero, G. & Villena, J. (2014). Immunobiotic *Lactobacilli* reduce viral-associated pulmonary damage through the modulation of inflammation-coagulation interactions. *International Immunopharmacology*. 19, 161 - 173.

Table 1: Growth of LAB (Log CFU/ml) at 0.3% bile salt

LAB Isolates	Bile tolerance in 0.3% bile salt		
	Viable counts (Log_{10} Cfu/ml)		
	Time (h) 0	4	Survival (%)
L. fermentum NBRC 15885	8.46 ± 0.01^{a}	7.32 ± 0.01^{b}	86.5
Leuconostoc mesenteroides LM	8.46 ± 0.01^{a}	7.93 ± 0.02^{a}	93.7
L. plantarum CIP 10315.1	7.38 ± 0.01^{e}	6.27 ± 0.02^{d}	85.0
L. plantarum NBRC 15891	7.32 ± 0.00^{f}	6.28 ± 0.02^{d}	85.8
L. parabuchneri LMG 11457	7.47 ± 0.01^{c}	7.31 ± 0.01^{b}	97.9
L. pentosus 124.3	7.44 ± 0.01^{d}	6.28 ± 0.02^{d}	84.4
L. brevis ATCC 14869	8.35 ± 0.01^{b}	7.06 ± 0.02^{c}	84.6

Values are means ± standard deviation of duplicate zones of inhibition

Means with the same superscript in the same column do not differ significantly (P>0.05)

LAB Isolates	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhimurium*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*	*Listeria monocytogenes*
L. fermentum NBRC 15885	24.00±1.53^{a} (S)	8.00±1.16^{e} (R)	15.00±1.00^{b} (I)	22.00±0.58^{de} (S)	15.00±1.00^{c} (I)	23.00±1.16^{cd} (S)
Leuconostoc mesenteroides LM	15.00±1.00^{f} (I)	15.00±1.00^{c} (I)	11.00±1.00^{d} (I)	19.00±0.58^{g} (I)	16.00±1.00^{b} (I)	24.00±1.00^{bc} (S)
L. plantarum CIP 10315.1	21.00±1.00^{cd} (S)	5.00±1.00^{f} (R)	7.00±1.00^{f} (R)	25.00±1.53^{abc} (S)	8.00±0.58^{e} (R)	25.00±1.16^{ab} (S)
L. plantarum NBRC 15891	18.30±0.58^{e} (I)	18.00±1.00^{b} (I)	12.00±1.00^{c} (I)	20.00±1.00^{fg} (S)	7.00±1.00^{e} (R)	21.00±1.00^{e} (S)
L. parabuchneri LMG 11457	22.00±1.00^{bc} (S)	22.00±1.53^{a} (S)	18.00±1.00^{a} (I)	25.00±1.00^{ab} (S)	9.00±1.53^{e} (R)	22.00±1.00^{de} (S)
L. pentosus 124.3	19.00±1.00^{d} (I)	9.00±0.58^{e} (R)	8.00±0.58^{e} (R)	21.00±1.53^{ef} (S)	18.00±1.00^{a} (I)	24.00±1.53^{abc} (S)

L. brevis ATCC 14869	23.00 ± 1.73^{b} (S)	10.00±1.00^{d} (I)	8.00±0.58^{e} (R)	23.00±1.00^{cde} (S)	15.00±0.58^{c} (I)	26.00±1.00^{a} (S)

Table 2: Antimicrobial resistance of the LAB against food borne pathogens

Values are means ± standard deviation of duplicate samples

Means that do not share same superscript in the same column are significantly ($P < 0.05$) different
S - Susceptibility, I - Intermediate; R - Resistance

	Zones of Inhibition (mm)

LAB Isolates	Gentamycin (10 µg/ml)	Ampiclox (30 µg/ml)	Cefuroxime (20 µg/ml)	Amoxacillin (30 µg/ml)	Ciproflocaxin (10 µg/ml)	Streptomycin (30 µg/ml)	Septrin (30 µg/ml)	Erythromycin (10 µg/ml)
L fermentum NBRC	8.00±1.00^{d} (R)	18.00±1.53^{c} (I)	21.00±1.53^{ab} (S)	21.00±1.53^{b} (S)	14.00±1.53^{c} (I)	17.00±2.00^{bc} (I)	15.00±1.00^{d} (I)	20.00±1.53^{d} (S)
Leuconostoc mesenteroides	9.00±1.00^{d} (R)	11.00±1.00^{de} (I)	18.00±0.58^{ab} (I)	13.00±1.53^{c} (I)	9.00±1.00^{e} (R)	14.00±1.53^{d} (I)	18.00±2.00^{c} (I)	21.00±1.00^{cd} (S)
L. plantarum CIP	15.00±1.53^{bc} (I)	23.00±5.69^{b} (S)	30.00±1.00^{ab} (S)	11.00±1.00^{d} (I)	14.00±.00^{c} (I)	19.00±1.00^{ab} (I)	21.00±1.53^{b} (S)	27.00±1.00^{a} (S)
L. plantarum NBRC	7.00±1.00^{e} (R)	11.00±1.00^{de} (I)	45.00±58.6^{a} (S)	9.00±1.00^{e} (R)	12.00±2.00^{d} (I)	9.00±1.00^{e} (R)	13.00±1.53^{de} (I)	14.00±1.53^{e} (I)
L. parabuchneri LMG 11457	15.00±1.00^{b} (I)	8.00±1.00^{e} (R)	21.00±1.53^{ab} (S)	9.00±1.00^{e} (R)	14.00±2.00^{c} (I)	14.00±1.53^{d} (I)	9.00±1.00^{e} (R)	13.00±1.00^{e} (I)
L. pentosus 124.3	21.00±1.00^{a} (S)	15.00±1.00^{cd} (I)	13.00±1.00^{b} (I)	20.00±1.00^{a} (S)	24.00±1.00^{a} (S)	20.00±0.53^{a} (S)	23.00±1.16^{a} (S)	26.00±0.58^{a} (S)
L. brevis ATCC 14869	16.00±1.00^{b} (I)	27.00±1.00^{a} (S)	21.00±1.53^{ab} (S)	13.00±1.00^{c} (I)	21.00±1.53^{b} (S)	19.00±1.53^{a} (I)	17.00±1.00^{cd} (I)	26.00±1.53^{ab} (S)

Table 3: Antibiotic susceptibility of LAB

Values are means ± standard deviation of duplicate samples

Means that do not share same superscript in the same column are significantly ($P < 0.05$) different
S - Susceptibility, I - Intermediate; R - Resistance

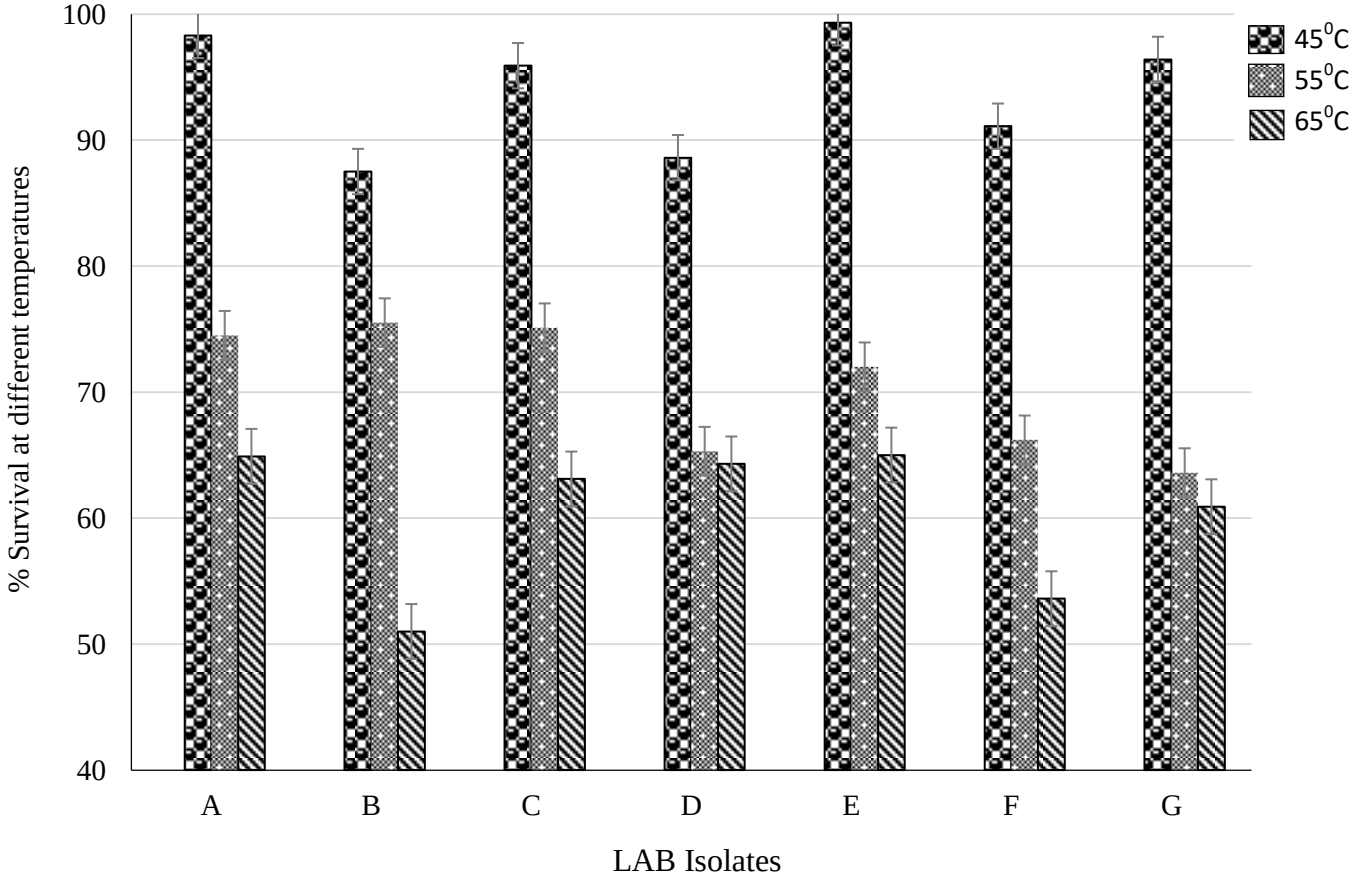

Figure 1: Growth of LAB at different temperature

A = *L. fermentum NBRC 15885;* B = *Leuconostoc mesenteroides LM*; C = *L. plantarum CIP 10315.1;* D = *L. plantarum NBRC 15891*; E = *L. parabuchneri LMG 11457*; F = *L. pentosus 124.3;* G = *L. brevis ATCC 14869*

Bars and error bars represent the % survival of the LAB strains at different temperatures and standard deviation of LAB trials
Bars with the same superscript for each temperature regime do not differ significantly (P > 0.05)

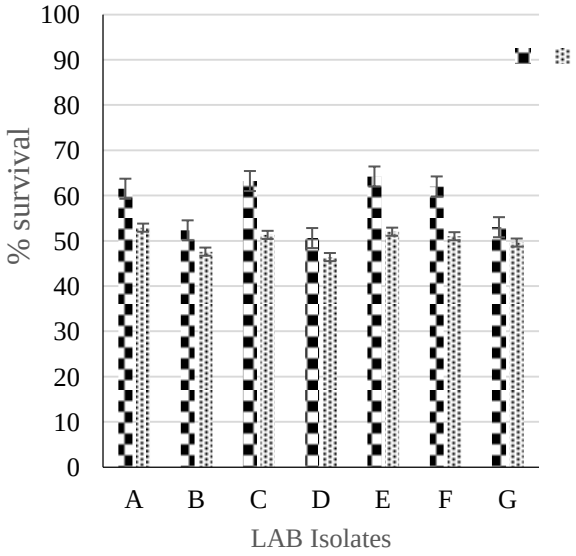

Figure 2a: Growth of LAB at pH 2.0

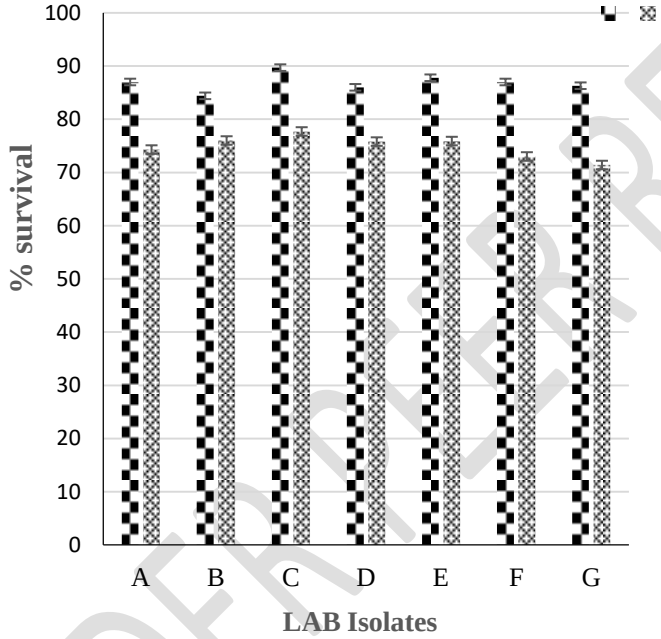

Figure 2b: Growth of LAB at pH 2.5

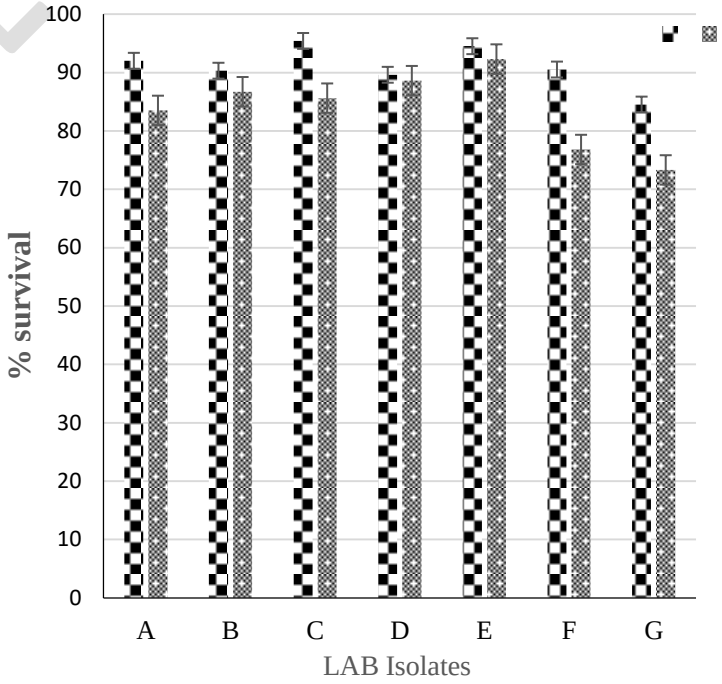

Figure 2c: Growth of LAB at pH 3.0

Figure 2: Growth of LAB at different pH and time

Legend:

A = *L. fermentum NBRC 15885;* B = *Leuconostoc mesenteroides LM*; C = *L. plantarum CIP 10315.1;* D = *L. plantarum NBRC 15891*; E = *L. parabuchneri LMG 11457*; F = *L. pentosus 124.3;* G = *L. brevis ATCC 14869*

Bar/error bars: % survival of the LAB strains at different pH and time

Bars with the same superscript in the same column do not differ significantly ($P > 0.05$)