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Full Length Research Paper

Production of freeze-dried lactic acid bacteria starter culture for cassava fermentation into *gari*

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Sixteen lactic acid bacteria, eight *Lactobacillus plantarum*, three *L. pentosus*, 2 *Weissella paramesenteroides*, two *L. fermemtum* and one *Leuc*onostoc *mesenteroides* ssp. *mesenteroides* were previously isolated from cassava fermentation and selected on the basis of their biochemical properties with a view to selecting appropriate starter cultures during cassava fermentation for *gari* production. In this study, the potential of these pre-selected strains as suitable freeze-dried cultures was evaluated. Their ability to tolerate the freeze-drying process was assessed by dehydration in a glycerol solution of increasing concentration, followed by staining with two fluorescent markers: rhodamine 123 and propydium iodide. Twelve strains that recovered more than 50% of their population value after visualisation on an epi-fluorescent microscope were produced in a bioreactor and freeze-dried. The technological characteristics identified after the freeze-drying process, were a high cell concentration or high survival rate. The ability of the freeze-dried strains to recover their acidification activity was evaluated through the determination of the pH, titratable acidity (% lactic acid/g Dry Weight) and cell count over 24 h on MRS broth. Ultimately, the strains *L. plantarum* VE36, G2/25, *L. fermentum* G2/10 and *W. paramesenteroides* LC11 were selected to be developed as freeze-dried starter cultures for *gari* production.

Key words: Freeze-drying, *gari*, lactic acid bacteria, fermentation, starter culture, cassava, developing countries, fermented food.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important food crop for millions of people in developing countries. In West Africa, it is traditionally processed into a range of products with various local names. One of the most popular foods derived from fermented cassava is *gari*, consumed by nearly 200 million people in West Africa (Okafor and Ejiofor, 1990). Its cheapness, longer shelf-life, lower bulk (compared with other cassava products) and ease of preparation for consumption account for its increasing popularity in the urban areas. The traditional production of *gari* includes: peeling, grating, fermentation at ambient temperature pressing, sieving and roasting ('garification'). It is now fairly well accepted that the flavour, quality, hygiene and safety of *gari* result from the

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Abbreviations: a_w, Water activity; **CFU,** colony forming unit; **DW,** dry cell weight; **LAB,** lactic acid bacteria; N_c, CFU/g DW before freeze-drying (at the end of centrifugation); N_0, CFU/g DW at the end of freeze-drying; N_{Lf}, proportion of viable cells before dehydration in glycerol solution; N_{Li}, proportion of viable cells after dehydration in glycerol solution; **P,** probability; **ΔpH**, Change in pH; **ΔLA**, Change in titratable acidity.

fermentative activities of lactic acid bacteria (LAB) and yeasts during the fermentation stage. Various investigations on the microbiology of cassava fermentation for *gari* production have been done. The dominant LAB belongs to *Lactobacillus plantarum* (the most predominant species), *Lactobacillus fermentum* and *Leuconostoc Weisella* (Kostinek et al., 2005; Oguntoyinbo, 2007; Okafor, 1977). Unfortunately, most of West Africa's fermented foods and beverages, including *gari*, are produced at household level or on a small industrial scale and therefore tend to be of varying quality and stability. Thus, the use of a preparation containing a large number of variable microorganisms is recommended, as this would lead to a rapid acidification of the product and inhibit the growth of spoilage and pathogenic bacteria (Holzapfel, 1997, 2002), as well as to a product with consistent quality.

The industrial use of LAB starter cultures in the food industry depends on the concentration and preservation technologies used, which are required to guarantee the long-term delivery of stable cultures in terms of viability and functional activity (Carvalho et al., 2002). The freeze-drying process has commonly been used for this purpose. The dehydration of LAB imposes environmental stress on the bacterial cells, such as freezing, drying, long-term exposure to low-water activities and rehydration. Microbial survival during this process depends on many factors, including the intrinsic resistance traits of the strains, initial concentration of microorganisms, growth conditions, drying medium and protective agents, freezing rate, storage conditions (temperature, atmosphere, relative humidity) and rehydration conditions (Andersen et al., 1999; Carvalho et al., 2002; Desmons et al., 1998; Morgan et al,. 2006). To date, relatively few LAB have been isolated from starchy fermented foods in Africa and used as starter cultures. Guiraud et al. (1998), Guiraud and Raimbault (1993) and Kimaryo et al. (2000) mentioned the use of the amylolytic *L. plantarum* as a starter culture during cassava fermentation for *gari* and *kivunde*, respectively. Okafor et al. (1998) inoculated *L. coryneformis* and *Saccharomyces* sp. as a starter culture in cassava mash for *gari* production. The survival of these microorganisms was studied when placed alone or mixed in different carriers (Okafor et al., 1999). As for *gari*, while the occurrence of the LAB starter culture was frequently reported, no detailed information was available on their production in the dried state.

Previously, eight *L. plantarum*, three *L. pentosus*, two *W. paramesenteroides*, two *L. fermemtum* and one *L. mesenteroides* ssp. *mesenteroides* strains had been isolated during cassava fermentation and they were then pre-selected as starter cultures for *gari* production on the basis of suitable technological characteristics (production of α-amylase, β-glucosidase, tannase, antimicrobials, and fermentation of raffinose and/or stachyose) and their ability to ferment cassava (based on the acidification rate during cassava fermentation) (Kostinek et al., 2007). The aim of this study was to evaluate the potential of these pre-selected strains for use as suitable freeze-dried starter cultures during cassava fermentation for *gari* production. For this purpose, the ability of the 16 strains to tolerate the freeze-drying process was first assessed by dehydration in a glycerol solution of increasing concentration, followed by staining with fluorescent markers. The ability of the surviving strains (more than 50% survival rate) to provide a freeze-dried powder with a high cell concentration (survival rate) and to grow on MRS broth after dehydration (rapid acidification, high growth) was evaluated. This study formed part of an EU project aimed at improving the quality and nutritional value of *gari* through the use of starter cultures and fortification with soybean and coconut milk.

MATERIALS AND METHODS

Microorganisms and preparation of the seed bank

The LAB *L. plantarum* G2/11, G2/25, G3/29, VE20, VE36, VE65b, VE82 and Lb50, *L. pentosus* G5/18, Lb61 and Lb68, *W. paramesenteroides* LC11 and LC18, *L. fermentum* G2/10 and G3/5, and *Leuc. mesenteroides* ssp. *mesenteroides* KMROG2 used in this study were provided by the Federal Research Centre for Nutrition, Institute of Hygiene and Toxicology (Karlsruhe, Germany). Each strain was inoculated in MRS broth and incubated at 30°C for 18 h. The cells obtained after centrifugation (2500 ×*g*, 20 min) were maintained in 50% (v/v) glycerol and frozen at -80°C.

Assessment of ability to tolerate dehydration using the fluorescence technique

The ability of the strains to tolerate the freeze-drying process was assessed by counting dead and viable cells under an epi-fluorescent microscope after dehydration with a glycerol solution of increasing concentration and staining with fluorescent markers. Two fluorescent dead/live markers, 1.25 mM rhodamine 123 and 1.25 mM propydium iodide, were used to stain cells, following the method described by Sow and Thonart (2003). Some 50 µl of each seed bank was inoculated in 150 ml MRS broth and incubated at 30°C for 17 h. The cell pellets obtained after centrifugation (2500 ×*g*, 20 min) were re-suspended in 1 ml peptone salt water (final concentration 10^9 cfu/ml) and dehydrated in a graded glycerol solution as described here; 1 ml of 90% (w/w) glycerol was added to the cell suspension, homogenised and allowed to stand for 15 min at room temperature. The same procedure was repeated four times and the cell suspension obtained was concentrated by centrifugation at 3500 ×*g* for 3 min. Cell suspension without glycerol was used as the control. Epi-fluorescence examination was made after staining 50 µl of the obtained cell suspension with 5 µl of each marker for 5 min in the dark. After centrifugation at 3500 ×*g* for 30 s, cell suspensions were observed twice and re-suspended in 100 or 200 µl of peptone salt water for dehydrated cells or the control, respectively. Counts and visualisation were carried out using a transmitted light microscope (Axioscop2.mot, ZEISS) equipped with an epi-fluorescent system that had a HAL 100 halogen illuminator for transmitted-light illumination. The exciting filter was set at 560 and 480 nm and the barrier filter at 631 and 527 nm, for propydium iodide and rhodamine 123, respectively. Dehydration and staining were performed in duplicate for each strain. The number of dead or viable cells was calculated by counting cells at 100 × magnification under the microscope during epi-fluorescence. Two readings were carried out for each sample and the average number of cells (dead and viable) was recorded.

Production and freeze-drying

Development of inocula

Flasks containing 300 ml MRS medium were inoculated from 50 µl of the seed bank. The flasks were incubated on a rotary shaker at 30°C and 75 rpm for 15 h. Flasks of 1 l containing MRS medium were inoculated from the inocula of 300 ml and incubated in a rotary shaker at 30°C and 75 rpm for 10 h.

Fermentation in a 20 L bioreactor

A 20 l bioreactor (16 l effective volume) was inoculated from a flask of 1 L that had been previously prepared (see inocula section, above). Agitation was performed at a speed of 80 rpm to keep the fermentation broth homogeneous. The temperature and pH were set to 37°C or 30°C and 7.0 or 6.0 with 4N KOH for *L. plantarum* and *L. pentosus* strains or *W. paramesenteroides*, *L. fermentum* and *L. mesenteroides* strains, respectively. No control of oxygen concentration was performed during the fermentation. All fermentation was done in duplicate and the average values recorded.

Concentration, treatment and freeze-drying

The culture was stopped after 17 h during the early stationary stage, cooled down to 4°C by the circulation of cold water in the double jacket of the bioreactor and then concentrated 20 times by centrifugation (3000 ×*g*, 30 min). The cell suspension was supplemented with 2% (w/w) and 5% (w/w) maltodextrin as protective compounds. The cells were freeze-dried in a Low freeze-drier (Leybold, Belgium) with a standard program by increasing the temperature gradually from -45°C to 25°C at 0.9 mbar pressure (30 h), followed by 15 h at 0.15 mbar. Freeze-dried powders were stored in aluminium foil packets which were vacuum-sealed and then stored at -20°C until further analysis.

Analytical methods

Dry cell weight and water activity determinations

The dry cell weights of the dried powders were determined at the end of freeze-drying after drying at 105°C until constant weight was achieved. The water activities (a_w) of the glycerol solution and dried samples after freeze-drying were measured with a Novasina (Novasina, Pfäffikon, Switzerland) water activity meter.

Survival

The viable counts were obtained using the plate count method after 48 h at 30°C with MRS-agar medium (pH 7.0 or 6.0 for *L. plantarum* and *L. pentosus* strains or *W. paramesenteroides*, *L. fermentum* and *L. mesenteroides* strains, respectively). The percentage survival after staining with the fluorescent dead/live markers (Rhodamine 123 and propydium iodide) was calculated as follows: Survival (%) = $100 \times N_{Lf}/N_{Li}$, where N_{Li} is the proportion of viable cells after dehydration in a glycerol solution and N_{Lf} is the proportion of viable cells before dehydration in a glycerol solution. The proportion of viable cells was determined by the ratio between green cells and green cells + red cells.

The percentage survival of the strains after the freeze-drying process was expressed as follows: Survival (%) = $N_0/N_c \times 100$, where N_0 is the CFU/g DW at the end of freeze-drying and N_c is the CFU/g DW before freeze-drying (at the end of centrifugation).

Acidification activity

Acidification was carried out at 30°C in 150 ml MRS broth (pH 7) inoculated with 1% of 10^7 cfu/g DW of the freeze-dried sample. The pH and total titratable acidity (% lactic acid/g DW) were determined in duplicate after 8, 16 and 24 h, using the AOAC method (1997). Some 10 ml of distilled water was homogenised with 10 ml of the inoculated broth, and the pH was measured. The titratable acidity was obtained by titration with 0.1 N NaOH, using 1% phenolphthalein as the indicator. Cell counts were obtained after 16 h culture using the plate count method.

Data analysis

The pH and titratable acidity after 16 h culture were expressed as change in pH (ΔpH) and titratable acidity (ΔLA) compared with those obtained immediately after freeze-drying. The production in the bioreactor was done in duplicate. Data were compared using Tukey's honestly significant difference (Statistica 8.0, StatSoft Inc., 2007). The level of significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Assessment of desiccation tolerance of lactic acid bacteria

The ability of the 16 strains to tolerate the freeze-drying process was assessed by counting dead and viable cells under an epi-fluorescent microscope after dehydration in a glycerol solution of increasing concentration (from 1 to 5 ml) and staining with two fluorescent markers (rhodamine 123 and propydium iodide). The a_w was measured after each addition of glycerol, respectively (Figure 1). An increase in glycerol concentration was associated with a decrease in the a_w from 0.8 to 0.3. The impact of the decrease in the a_w on the viability of the strains was studied after staining with the fluorescent markers and was compared with that of the control (cells without dehydration). Figure 2 A, B, C shows the result of the visualisation under an epi-fluorescent microscope for *L. plantarum* VE36, *L. fermentum* G3/5 or *L. pentosus* G5/18. With the two fluorescent markers, living cells appeared green because they tended to accumulate the rhodamine 123 (positively charged) in the cytoplasm (negatively charged). It is now well established that a bacterial cell with an intact energy state is metabolically active, and therefore viable. The dead cells appeared red because their damaged cytoplasmic membrane left propydium iodide, which was fixed on the nucleic acids. Propydium iodide and rhodamine123 are most commonly used as indicators for cell death or cells with compromised membrane and cells with an intact membrane (that is, membrane potential), respectively (Rault et al., 2008; Sow and Thonart, 2003). The change in the a_w in the environment of the bacteria had a significant effect on their viability, and this varied from one strain to another, as shown in Table 1. Most of the strains (12 out of 16) survived or recovered more than 50% of their population value, whereas four strains (*L. plantarum* G3/29, Lb50, *L.*

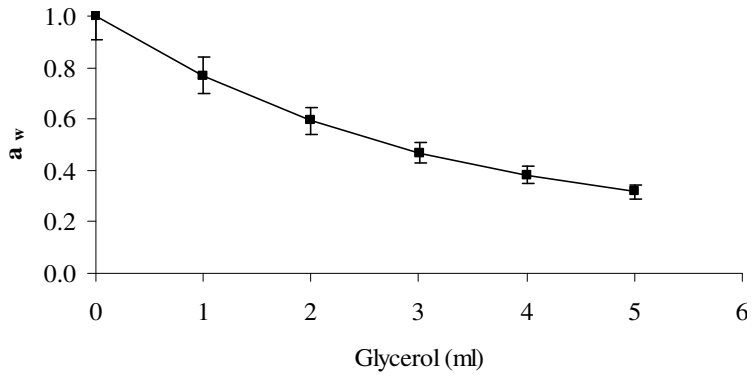

Figure 1. Interrelationships between the water activity (a_w) and glycerol concentration. Values are means ± standard deviation (SD, n = 4).

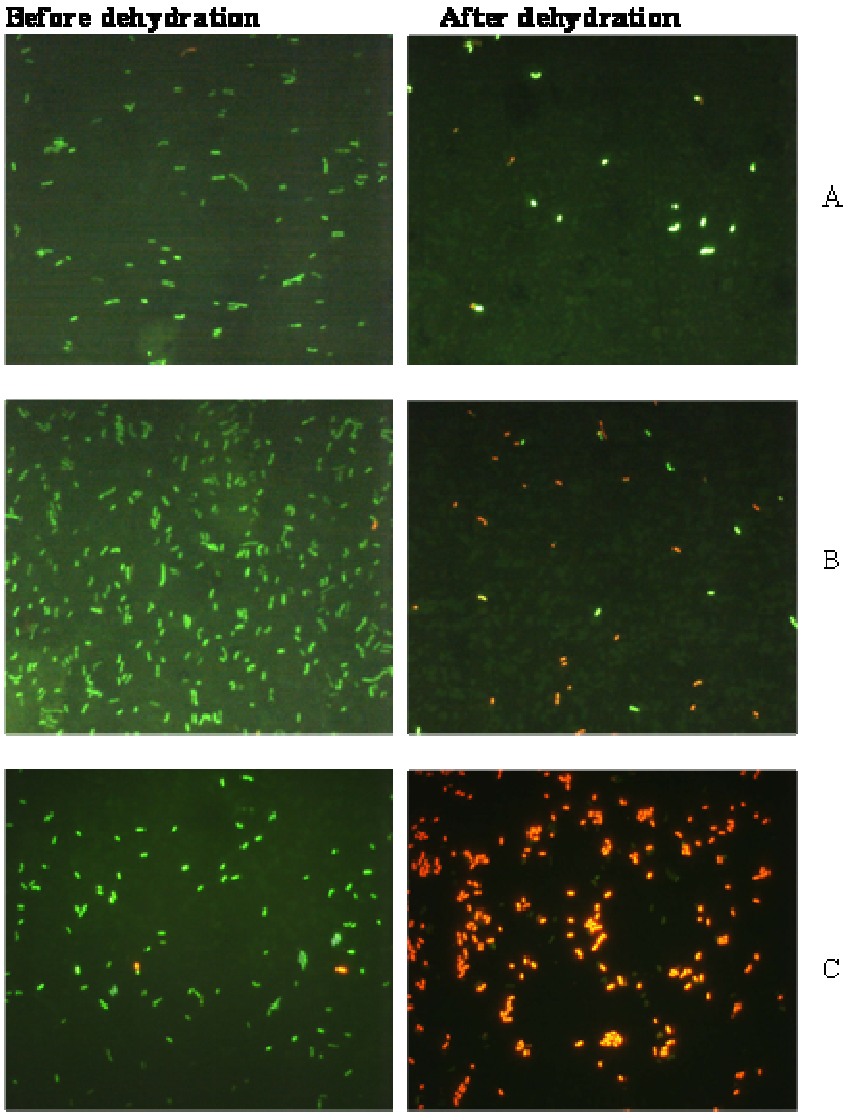

Figure 2. Epi-fluorescent microscope visualisation of *L. plantarum* VE36 (A), *L. fermentum* G3/5 (B) or *L. pentosus* G5/18 (C) before and after dehydration in a glycerol solution of increasing concentration, followed by staining with two fluorescent markers: rhodamine123 (green cells) and propydium iodide (red cells).

Table 1. The survival after dehydration in glycerol solution of increasing concentration and staining with the fluorescent markers [a]

Strains	Proportion of viable cells (N_L)[b]		Survival (%)
	before dehydration	After dehydration	
L. plantarum VE82	0.98 ± 0.03	0.50 ± 0.08	51.1 ± 8.9 a
L. plantarum VE 65b	0.99 ± 0.01	0.51 ± 0.08	51.2 ± 4.7 a
L. plantarum VE 20	1.00 ± 0.01	0.81 ± 0.08	81.0 ± 9.6 bc
L. plantarum VE 36	1.00 ± 0.05	0.71 ± 0.05	71.0 ± 5.5 c
L. plantarum G2/11	0.98 ± 0.01	0.80 ± 0.05	81.8 ± 6.2 cb
L. plantarum G2/25	0.97 ± 0.01	0.91 ± 0.05	93.1 ± 5.0 b
L. plantarum G3/29	0.98 ± 0.03	0.17 ± 0.07	17.1 ± 5.5 d
L. plantarum Lb50	1.00 ± 0.02	0.10 ± 0.04	10.2 ± 4.4 d
L. pentosus G5/18	0.98 ± 0.02	0.07 ± 0.02	6.7 ± 2.1 d
L. pentosus Lb61	0.99 ± 0.01	0.60 ± 0.05	60.3 ± 5.4 ac
L. pentosus Lb68	1.00 ± 0.02	0.14 ± 0.03	14.0 ± 3.5 d
W. paramesenteroides LC11	0.99 ± 0.02	0.72 ± 0.06	73.0 ± 6.7 c
W. paramesenteroides LC18	0.99 ± 0.01	0.70 ± 0.02	70.5 ± 3.0 c
Leuc. mesenteroides ssp. mesentroides KMROG2	0.99 ± 0.01	0.50 ± 0.07	50.2 ± 7.5 a
L. fermentum G3/5	0.99 ± 0.02	0.54 ± 0.06	54.6 ± 6.6 a
L. fermentum G2/10	0.96 ± 0.02	0.78 ± 0.03	82.0 ± 3.9 c

pentosus G5/18, Lb68) had very low survival rates (≤10%). In an earlier study, Poirier et al. (1997) used a progressive water activity decrease in a water-glycerol solution to test the mechanical resistance of the cell wall for *L. plantarum* and *Leuc. mesenteroides*. Sow and Thonart (2003) indicated a loss of viability of 40-100% for LAB after 7 days of dehydration in a water glycerol solution with an a_w of 0.17. As the freeze-drying process imposes an exposure to low-water activities on the bacterial cells, we decided to select the strains with more than 50% survival for production in the bioreactor. The results obtained and reported here are only approximate, since the final a_w of the water glycerol solution in our study (0.3) was higher than that obtained generally for freeze-dried lactic acid powders (0.1-0.2) (Cheftel and Cheftel, 1980). However, the dehydration technique, followed by staining with fluorescent markers, remains a fast method for screening and predicting the desiccation tolerance of LAB (Sow and Thonart, 2003).

Survival of lactic acid bacteria after freeze-drying

The 12 selected strains were produced in the bioreactor, concentrated 20 times by centrifugation, supplemented with cryoprotectants (5% maltodextrin, 2% glycerol) and freeze-dried. Table 2 shows the viable counts during the process and the survival after the freeze-drying process. Freeze-drying proved effective in achieving high viable cells (> 10^9). The preservation of microorganisms by freeze-drying has been mentioned in relation to preserving high cell suspension that contains more than 10^8 cells/ml (Miyamoto-Shinohara et al., 2000). Various survival rates were obtained immediately after the freeze-drying. *L. plantarum* and *L. pentosus* strains survived or recovered more than 32% of their population value, whereas *Leuconostoc*, *Weissella* and *L. fermentum* strains had a low biomass yield (<16%). Our results accord with those reported by Vedamuthu (1994) who found that low survival rates after freeze-drying were obtained with *Leuconostoc* strains compared with *Lactococcus* or *Lactobacillus* strains in the same conditions. Freezing and drying from the frozen state caused a decrease in viability (Castro et al., 1997). This effect was less evident in the strains *L. plantarum* VE36, G2/25 and *L. pentosus* LB61, probably because of the elimination of the most sensitive part of the cell population during the freezing stage. At the end of freeze-drying, the rate of dry matter in the lyophilized powders was nearly 95% (Table 2), with an a_w of 0.1 (data not shown). Béal and Corrieu (1994) and Ishibashi et al. (1985) showed that the stability of dried LAB during storage was better in the 0.1-0.2 a_w zone, with a dry-matter rate of almost 96%. The strains *L. plantarum* VE36, G2/25 and *L. pentosus* Lb61 could constitute a starter culture, or are of interest in cassava fermentation because of their ability to provide a freeze-dried powder with a high cell concentration.

Acidification activity

To evaluate the ability of the freeze-dried strains to

Table 2. Viable count during production, survival after freeze-drying and moisture content of the dried samples

Strains	Moisture content of the dried powder (%)	Cell count (cfu/g DW)		Survival (%)
		before freeze-drying	After freeze-drying	
L. plantarum VE82	4.3 ± 0.5 a	1.8×10^{12}	6.7×10^{11}	37.2 ± 5.0 ac
L. plantarum VE 65b	5.1 ± 0.3 a	1.5×10^{11}	6.2×10^{10}	41.3 ± 4.5 a
L. plantarum VE 20	5.0 ± 0.7 a	2.0×10^{12}	9.2×10^{11}	46.8 ± 2.9 a
L. plantarum VE 36	5.6 ± 0.2 a	7.2×10^{11}	6.9×10^{11}	97.3 ± 7.2 b
L. plantarum G2/11	5.3 ± 0.7 a	1.7×10^{1}	7.6×10^{10}	44.5 ± 1.9 a
L. plantarum G2/25	5.6 ± 0.1 a	8.1×10^{10}	6.5×10^{10}	79.9 ± 1.3 b
L. pentosus Lb61	5.5 ± 0.3 a	4.6×10^{10}	3.5×10^{10}	76.7 ± 6.5 b
W. paramesenteroides LC11	4.8 ± 0.2 a	2.6×10^{13}	4.9×10^{12}	18.0 ± 2.4 c
W. paramesenteroides LC18	5.3 ± 0.7 a	1.5×10^{13}	2.1×10^{12}	14.2 ± 3.9 c
Leuc. mesenteroides ssp. mesentroides KMROG2	5.0 ± 0.7 a	1.4×10^{13}	1.9×10^{12}	13.9 ± 4.2 c
L. fermentum G3/5	4.2 ± 0.5 a	3.5×10^{10}	3.8×10^{9}	11.3 ± 1.6 c
L. fermentum G2/10	5.1 ± 0.6 a	5.6×10^{12}	6.9×10^{11}	12.7 ± 3.0 c

Within the same column, means with different letters are significantly different $P < 0.05$ (Tukey HSD test, $n = 4$)

recover their acidification activity after dehydration, the pH and titratable acidity (% lactic acid/g DW) were measured and determined over 24 h on MRS broth, respectively. Figure 3 A, B, C shows the pH and titratable acidity for *L. plantarum* VE36, *W. paramesenteroides* LC11 and *L. pentosus* Lb61, respectively. The pH and the titratable acidity decreased and increased as the incubation time increased, respectively. After the 16 h incubation period, the pH and the titratable acidity were compared with those obtained immediately after freeze-drying and expressed as a change in pH (ΔpH) and acidification activity (ΔLA) (Table 3). The change in pH and titratable acidity after 16 h incubation were significantly higher for MRS broth inoculated with *L. plantarum* VE36, G2/25 and *L. fermentum* G2/10 strains than for those inoculated with the other strains ($P < 0.05$). For example, the change in pH or titratable acidity for MRS broth inoculated with *L. plantarum* G2/25 was 1.2- and 1.7-fold higher, respectively, than that inoculated with *L. pentosus* Lb61. After the 16 h incubation period, the cell count was significantly higher for MRS broth inoculated with the *L. plantarum* VE36, G2/25 and *L. fermentum* G2/10 strains than for those inoculated with the other strains ($P < 0.05$) (Table 3). An important characteristic for potential starter strains is their ability to acidify their environment rapidly, as the acid production and the accompanying decrease in pH are known to extend the lag phase of sensitive organisms, including food-borne pathogens (Smulders et al., 1986). Leroy and De Vuyst (2004) reported that the direct addition of a selected starter culture to raw materials resulted in dominance by the best-adapted strains. We conclude that the strains *L. plantarum* VE36, G2/25 and *L. fermentum* G2/10 could constitute starter cultures or are of interest in cassava fermentation because of their rapid acidification and high growth in MRS broth after dehydration.

Conclusion

In this study, the potential of 16 pre-selected LAB for use as suitable freeze-dried starter cultures during cassava fermentation for *gari* production was assessed. The strains, *L. plantarum* VE36, G2/25 and *L. pentosus* Lb61 were selected on the basis of their high cell concentration and high survival rate after freeze-drying. The strains *L. plantarum* VE36, G2/25 and *L. fermentum* G2/10 were shown to be capable of a rapid acidification and high growth in MRS broth after the dehydration process. In addition, the strains *L. plantarum* VE36 and *W. paramesenteroides* LC11 was previously selected on the basis of their stability over 2 months at 4°C in their dried state (Yao et al., 2008). All these strains was shown previously to be capable of fermenting the indigestible sugars stachyose and raffinose (Kostinek et al., 2007). They also showed presumptive bacteriocin activity and two of these presumptive bacteriocin-producing strains (*L. plantarum* VE36 and *L. fermentum* G2/10) were able to produce hydrogen peroxide. Ultimately, the strains *L. plantarum* VE36, G2/25, *L. fermentum* G2/10 and *W. paramesenteroides* LC11 were selected for development as starter cultures and are currently being evaluated, either alone or in combination, in soy-palm oil cassava mash fermentation for *gari* production in Bénin.

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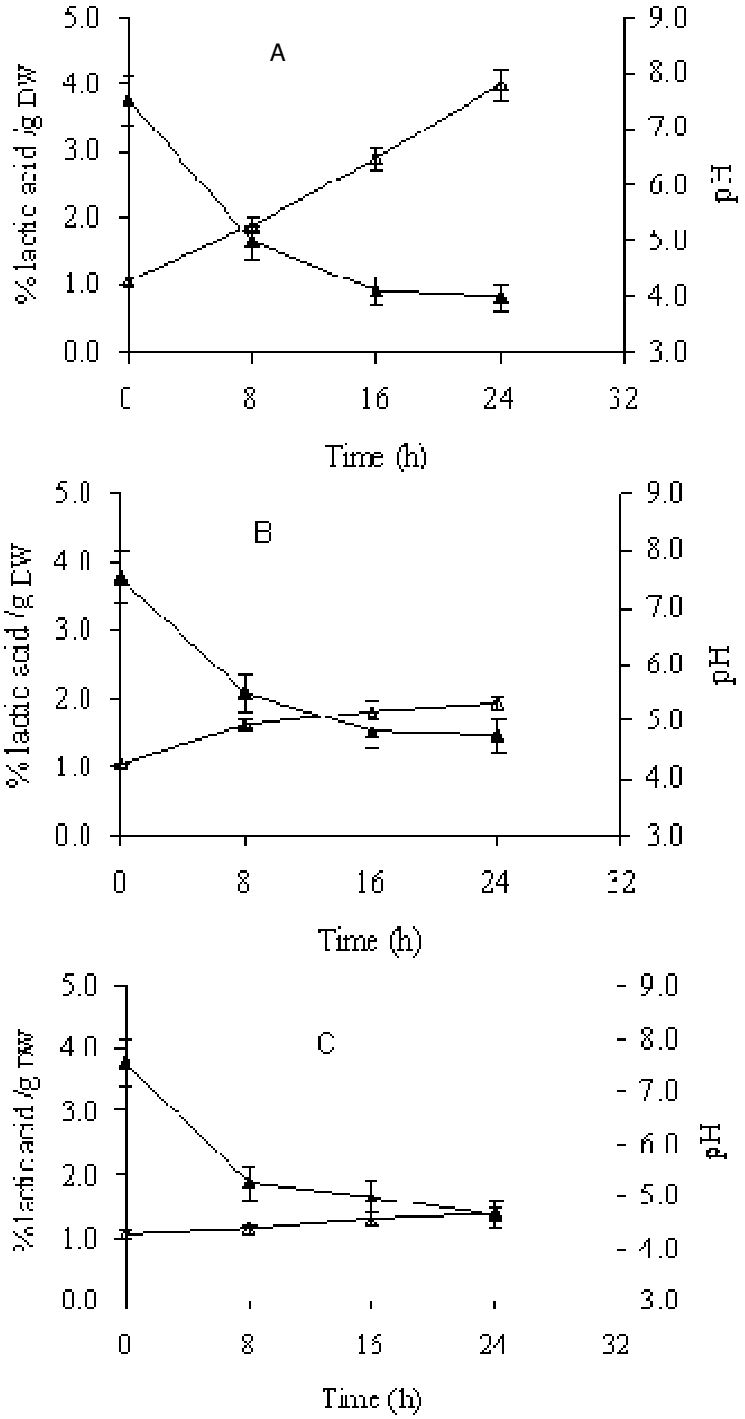

Figure 3. The pH (▲) and titratable acidity (% lactic acid/g DW) (Δ) of freeze-dried *L. plantarum* VE36 (A), *W. paramesenteroides* LC11 (B) or *L. pentosus* Lb61 (C). Values are presented as means ± SD (*n* = 4).

European Commission within the framework of the INCO-RTD Programme. The study was carried out partly under the project 'Improving the quality and nutritional status of *GARI* through the use of starter cultures and fortification with soybean, palm oil and coconut milk (ICA4-CT-2002-10058)'. The study does not necessarily reflect the project's views and does not anticipate the Commission's future policy in this area.

Table 3. The change in pH (ΔpH) or titratable acidity (Δ lactic acid) and viable count after 16 h incubation in MRS broth

Strains	ΔpH[a]	ΔLA[b] (% lactic acid)	Cell count (cfu/g DW)
L. plantarum VE82	2.84 ± 0.06 ac	1.05 ± 0.16 a	5.1×10^8 a
L. plantarum VE 65b	2.90 ± 0.11 abc	0.85 ± 0.03 ace	2.5×10^8 a
L. plantarum VE 20	2.92 ± 0.24 abc	1.03 ± 0.18 ae	4.3×10^8 a
L. plantarum VE 36	3.43 ± 0.07 bcd	1.82 ± 0.12 b	8.5×10^8 ad
L. plantarum G2/11	2.54 ± 0.04 a	0.44 ± 0.06 ce	1.6×10^8a
L. plantarum G2/25	3.20 ± 0.23 c	2.24 ± 0.07 b	6.1×10^9 b
L. pentosus Lb61	2.57 ± 0.14 a	0.23 ± 0.04 ce	2.3×10^7 a
W. paramesenteroides LC11	2.71 ± 0.17 a	0.77 ± 0.14 ace	4.3×10^8 a
W. paramesenteroides LC18	2.86 ± 0.08 a	0.72 ± 0.09 ace	3.2×10^8 a
Leuc. mesenteroides ssp. mesentroides KMROG2	2.61 ± 0.13 a	1.01 ± 0.07 ae	2.3×10^8 a
L. fermentum G3/5	2.72 ± 0.06 a	0.56 ± 0.05 e	4.4×10^7 c
L. fermentum G2/10	3.84 ± 0.05 d	1.51 ± 0.10 b	1.1×10^9 d

Within the same column, means with different letters are significantly different $P < 0.05$ (Tukey HSD test, $n = 4$)
[a] values compared with those obtained immediately after freeze-drying. Initially, pH value of MRS broth was 7.5.
[b] values compared with those obtained immediately after freeze-drying. Initially, titratable acidity of MRS broth was approximately 1.06 % lactic acid/g DW ($n = 12$).

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