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ORIGINAL ARTICLE

Development of starter culture for improved processing of Lafun, an African fermented cassava food product

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Abstract

Aims: To select appropriate micro-organisms to be used as starter culture for reliable and reproducible fermentation of Lafun.
Methods and Results: A total of 22 cultures consisting of yeast, lactic acid bacteria (LAB) and *Bacillus cereus* strains predominant in traditionally fermented cassava during Lafun processing were tested as potential starter cultures. In an initial screening, *Saccharomyces cerevisiae* 2Y48P22, *Lactobacillus fermentum* 2L48P21, *Lactobacillus plantarum* 1L48P35 and *B. cereus* 2B24P31 were found to be the most promising of the cultures and were subsequently tested in different combinations as mixed starter cultures to ferment submerged cassava roots. *Saccharomyces cerevisiae*, inoculated singly or combined with *B. cereus*, gave the softest cassava root after 48 h of fermentation according to determination of compression profile and stress at fracture. Overall, sensory quality testing showed that Lafun obtained from *S. cerevisiae*-fermented cassava gave the most preferred stiff porridge. *Saccharomyces cerevisiae* 2Y48P22 showed pectinase production in a model system.
Conclusions: The results suggest that *S. cerevisiae* 2Y48P22 is the most efficient organism for cassava softening during the fermentation. Therefore, it could be combined with LAB and used as starter for Lafun processing.
Significance and Impact of the Study: Starter cultures are made available for controlled fermentation of Lafun.

Introduction

Cassava roots are processed in several ways to avoid their postharvest deterioration (Westby 2002), to reduce the toxicity of the roots used as food (Oyewole 1995; Amoa-Awua *et al.* 1996), to improve the palatability of the derived products (Oyewole and Odunfa 1992; Nout and Sarkar 1999) and to increase the trading value of cassava. Several cassava processing methods including fermentation of the root are used in Africa. Fermentation has been shown to be a suitable method to enhance the safety, organoleptic and nutritional quality of many cassava-derived foods (Cooke *et al.* 1987; Nout and Motarjemi 1997; Oyewole 1997; Caplice and Fitzgerald 1999; Kimaryo *et al.* 2000). However, in Africa, fermentation of cassava often takes place as a spontaneous process although traditional inocula are used to ferment cassava while processing traditional foods such as Agbelima (Amoa-Awua *et al.* 1996, 1997), Attiéké (Coulin *et al.* 2006) and Lafun (Padonou *et al.* 2009a). The quality of the end product thus varies considerably (Padonou *et al.* 2009a).

Lafun, as it is called in Benin and Nigeria, is an African cassava fermented food product obtained by soaking peeled cassava chunks in water, at ambient temperature (28–32°C) for 2–5 days. The cassava chunks are later sun-dried and milled. The flour is used to cook a stiff porridge (Oka) often consumed with various stews. During the fermentation process, different biochemical changes occur such as degradation of cyanogenic compounds; formation of flavour compounds; and softening of the roots (Oyewole and Odunfa 1988, 1990, 1992; Ampe and Brauman 1995). The degree of root softening is the most important criterion to determine the end of Lafun fermentation (Oyewole and Odunfa 1988; Padonou *et al.* 2009b). In this respect, the activities of pectinolytic enzymes such as pectin methylesterase and pectate lyase have been reported to occur during cassava fermentation (Ampe and Brauman 1995; Brauman *et al.* 1996). These enzymes originate from micro-organisms associated with the fermentation process (Okafor *et al.* 1984; Ampe and Brauman 1995). However, like other traditional fermented products, Lafun is spontaneously fermented and its microbiological composition is of mixed nature (Padonou *et al.* 2009a,b). In a previous study, the predominant micro-organisms associated with traditional Lafun fermentation have been isolated and identified (Padonou *et al.* 2009b). The microbiota identified included several yeasts species with predominance of *Saccharomyces cerevisiae*, *Pichia scutulata* and *Kluyveromyces marxianus*; lactic acid bacteria (LAB) such as *Lactobacillus fermentum* and *Lactobacillus plantarum*; and sporeformers – mainly *Bacillus cereus*. The yeasts, the LAB and *B. cereus* were detected at levels of 3 $\log_{10}$, 5 $\log_{10}$ and 5–6 $\log_{10}$ CFU g^{-1}, respectively, at the beginning of the spontaneous fermentation. While yeasts and LAB were reported to grow reaching levels of 5–6 $\log_{10}$ and 9 $\log_{10}$ CFU g^{-1}, respectively, *B. cereus* were not detected at the end of the fermentation process. The present work was undertaken to assess the role of each of these predominant micro-organisms in root softening and the overall organoleptic quality and to develop a suitable starter culture for the standardized production of Lafun.

Materials and methods

Stages and localities of the experiment

The present work was carried out in three stages. First, fermentations with single starter cultures were undertaken that led to the selection of the microbial strains to be used the next stage for mixed starter culture fermentation. The last stage consisted of Lafun processing by the use of selected microbial strains, sensorial analyses and enzymes assays. The first and second stages and the related physical and microbiological analyses were carried out at the Faculty of Life Science, University of Copenhagen (Denmark), while the last stage was carried out at Faculté des Sciences Agronomiques, Université d'Abomey-Calavi (Bénin). The experiment was carried out at each stage on three different occasions.

Root samples preparation

Cylindrical fresh cassava root pieces (diameter 1·6 cm; length 2·5 cm) were cut from the flesh. These pieces were decontaminated by soaking in ethanol 70% (v/v) for 15 min as described by Okolie and Ugochukwu (1988) and subsequently washed in sterile deionized and purified water (milli-Q water; Millipore Corp., Billerica, MA). For each experimental sample, ten cylindrical cassava pieces (totalling about 50–55 g) were soaked with 100 ml sterile milli-Q water in a 250-ml conical flask and inoculated for fermentations under laboratory conditions with single and mixed cultures.

Micro-organisms included in the study

A total of 22 strains of yeasts, LAB and *B. cereus* isolated and identified during a previous study and representing predominant micro-organisms associated with spontaneous Lafun fermentation (Padonou *et al.* 2009b) were included. Selection within species was based on differences in their rep-PCR profiles that determined the different groups indicated in the aforementioned study. The strains are listed in Table 1.

Inocula preparation and inoculation of the soaked cassava pieces

Purified isolates of the selected yeast strains stored at −80°C in Malt Yeast Glucose Peptone (MYGP) broth [3 g l^{-1}Malt Extract (Difco, Becton Dickinson & Co., USA), 3 g l^{-1} Yeast Extract (Difco), 10 g l^{-1} Glucose (Merck KGaA, Darmstadt, Germany), 5 g l^{-1} Bacto Peptone (Difco)] containing 20% (w/w) glycerol were streaked onto MYGP agar plates [20 g l^{-1} Agar (Difco), pH adjusted to 7·0]. The plates were incubated at 25°C for 48 h. A single colony was subcultured in MYGP broth at 25°C for 24 h. The number of cells per ml in this culture was counted by microscopy (×400, Olympus BX 40, Olympus Optical Co. Ltd, Tokyo, Japan) with a Neubauer counting chamber (Neubauer, Germany). One ml of the culture was centrifuged for 10 min at 3000***g***, the pellet washed in 1 ml sterile diluent [8·5 g NaCl (Merck) and 5 g Bacto Peptone (Difco) in 1000 ml milli-Q water, pH = 7·0]. The pellet was suspended again in 1 ml sterile diluent, and the suspension was inoculated in the soaked cassava pieces with a final concentration of about 3 $\log_{10}$ CFU g^{-1}. The initial microbial load was checked by the plate counting method described by Amoa-Awua *et al.* (1997) on MYGP agar plates, 100 mg l^{-1} Chloramphenicol (Boehringer Mannheim GmbH, Germany) and 50 mg l^{-1} Chlortetracycline (Sigma, St Louis, MO, USA) added (results not shown).

Similarly, purified isolates of selected LAB strains stored at −80°C in MRS (de Man, Rogosa and Sharp) broth (Merck) containing 20% (w/w) glycerol were streaked onto MRS agar (Merck) plates and incubated anaerobically (Anaerocult® A, Merck) at 30°C for 48 h.

Table 1 Screened strains and their effect on pH and texture of cassava determined as stress at fracture after 48 h fermentation time

Inoculated strains†	pH of the fermented cassava‡ 0 h	48 h	Stress at fracture of the cassava pieces at 48 h (MPa)§
Saccharomyces cerevisiae group			
2Y24P33	6·34	5·75 ± 0·21[a]	1·04 ± 0·08[a]
2Y48P22*	6·30	5·64 ± 0·14[a]	0·55 ± 0·09[b]
1Y48P31	6·35	5·65 ± 0·17[a]	0·87 ± 0·25[ab]
Pichia scutulata group			
2Y48P21*	6·29	5·62 ± 0·16[b]	1·14 ± 0·30[c]
2Y48P33	6·36	5·72 ± 0·09[b]	1·22 ± 0·29[c]
Kluyveromyces marxianus group			
2Y24P12*	6·32	5·64 ± 0·13[c]	0·99 ± 0·17[d]
2Y24P14*	6·33	5·68 ± 0·15[c]	1·06 ± 0·14[d]
Lactobacillus plantarum group			
1L48P35*	6·32	3·92 ± 0·07[d]	1·03 ± 0·21[e]
1L48P15	6·36	4·27 ± 0·10[e]	0·89 ± 0·08[e]
2L48P11	6·37	3·92 ± 0·11[d]	1·09 ± 0·17[e]
2L0P14	6·30	3·92 ± 0·08[d]	1·17 ± 0·24[e]
2L0P31	6·34	3·98 ± 0·12[d]	0·98 ± 0·06[e]
Lactobacillus fermentum group			
2L24P32	6·28	4·45 ± 0·10[fg]	0·88 ± 0·09[f]
2L24P23	6·35	4·47 ± 0·06[fg]	1·21 ± 0·08[f]
2L48P25	6·31	4·42 ± 0·05[fg]	1·17 ± 0·23[f]
2L48P21*	6·35	4·34 ± 0·03[f]	1·02 ± 0·24[f]
1L48P32	6·30	4·56 ± 0·12[g]	1·15 ± 0·10[f]
Bacillus cereus group			
1B24P32	6·34	5·39 ± 0·13[h]	1·05 ± 0·22[g]
2B24P31*	6·36	5·34 ± 0·11[h]	1·08 ± 0·10[g]
2B0P11	6·29	5·35 ± 0·07[h]	0·94 ± 0·30[g]
1B0P31	6·32	5·32 ± 0·10[h]	1·08 ± 0·08[g]
2B0P21	6·36	5·44 ± 0·18[h]	1·01 ± 0·22[g]

†Numbering according to Padonou *et al.* (2009b). Sequenced 16S rRNA gene strains were marked with * and sequences were released in GenBank database.
‡Results given as mean of data from three fermentations. Data with different superscript letters within a group of micro-organisms in a column are significantly different ($P < 5\%$).
§Results given as mean of data from three fermentations ± SD. Data with different superscript letters within a group of micro-organisms in a column are significantly different ($P < 5\%$).

A colony was picked and grown in 10 ml MRS broth at 30°C for 24 h. A volume of 0·1 ml was transferred into a new 10 ml MRS broth and incubated under the same conditions. The number of cells was counted and the inoculum prepared as described earlier. The inoculum was added to the soaked cassava pieces giving a final concentration of about 5 $\log_{10}$ CFU g^{-1} checked by MRS agar plates counting (results not shown).

For *B. cereus* strains, the purified isolates stored under the same conditions [−80°C in Nutrient Broth (Difco) with 20% (w/w) glycerol] were streaked onto Nutrient agar (Difco) plates and incubated at 37°C for 18 h. One colony of each isolate was subcultured in Nutrient broth at 37°C for 24 h. The number of cells was counted and the inoculum prepared as described elsewhere. The final concentration in the submerged cassava preparation was about 6 $\log_{10}$ CFU g^{-1} checked by Nutrient agar plates counting (results not shown).

Screening for selection of strains to be tested in mixed culture fermentation and Lafun processing

Fermentations with single cultures

A screening by fermentations with single-strain starter cultures was conducted with the purpose of selecting among the aforementioned strains the most promising to be used as mixed starter culture. The concentrations of the inocula at the onset of the fermentations mentioned earlier corresponded to the levels reported at the beginning of spontaneous fermentation of cassava for Lafun production (Padonou *et al.* 2009b). A control preparation made up of cassava root pieces in 100 ml sterile milli-Q water remained noninoculated. The flasks were manually shaked for 10 s and incubated at 30°C for 48 h without shaking.

Examination of the microbiota of the fermenting media inoculated by single starter cultures

A loopful liquid from 48-h fermenting cassava flasks was streaked onto agar plates (MYGP agar for flasks inoculated by yeasts and MRS agar and Nutrient agar for flasks inoculated by LAB and *B. cereus* strains, respectively). After incubation at 30°C for 3–4 days, all colony types were examined by macroscopic observation, catalase production, Gram test (Gregersen 1978) and cell morphology (×1000, Olympus BX 40; Olympus Optical Co. Ltd).

pH measurement

Ten grams (about two cylindrical pieces) of 0 h, 24 h and 48 h fermenting cassava was each aseptically taken from the flasks, crushed and homogenized in 40 ml milli-Q water. The pHs of the homogenates were measured using a digital pH/ion meter (PHM 95, Radiometer A/S, Brønshøj, Denmark).

Texture of the fermented cassava

After 48 h fermentation time, the softening of five to six cylindrical fermented cassava pieces from each flask was assessed by the means of an Instron uniaxial compression instrument (Instron model 5564; Instron Corp., Canton, MA). Hencky strain ε (intensity of the deformation because of the compression) and the stress σ applied until the fracture of the cassava pieces were recorded (Hencky 1931; Steffe 1996).

Mixed starter culture fermentations

Softening of the cassava pieces after 48 h of single-strain fermentation assessed by the lower stress at fracture was the main criterion for selecting the most promising strains to be used for the mixed fermentations trials. In addition, the pH of the fermented cassava was used as criterion as low pH improves the safety of the final product. Based on these criteria, the *Saccharomyces cerevisiae* 2Y48P22, the *Lact. fermentum* 2L48P21, the *Lact. plantarum* 1L48P35 and the *B. cereus* 2B24P31 were selected and used in several combinations as inocula. pH and stress at fracture were assessed as described previously.

Microbiological analyses

From each flask containing soaked cassava roots, two pieces (about 10 g) were aseptically taken at 0 and 48 h fermentation time, crushed and homogenized in a stomacher (Seward Stomacher 400C Lab Blender, Worthing, W. Sussex, UK) with 90 ml sterile diluent made up of 0·1% BactoPeptone (Difco) and 0·8% NaCl in milli Q water. Serial tenfold dilutions were prepared. A volume of 0·1 ml of the prepared dilutions was spread onto plates. Yeasts were enumerated on MYGP agar, added the aforementioned antibiotics. They were incubated at 25°C for 3–5 days, and colony forming units (CFU) were recorded. LAB were grown on MRS agar (Merck) containing 0·05% Cycloheximide (ICN Biomedicals Inc., Aurora, OH, USA) to avoid fungal growth. The plates were incubated under anaerobic conditions at 30°C for 3 days and cfu recorded. *Bacillus* spp. were grown and enumerated on Nutrient agar (Difco) with 0·05% Cycloheximide (ICN Biomedicals) added and incubated at 37°C for 3 days. Colonies were also examined by microscopy and bacteria tested for Gram reaction and catalase production.

Use of starter cultures for Lafun processing

Two kilograms peeled cassava pieces roughly sterilized and treated as described previously were fermented during 48 h in sterile jars at ambient temperature (28–32°C) using 2Y48P22, 2Y48P22 + 2B24P31, 2Y48P22 + 1L48P35 and 2Y48P22 + 2L48P21 as starter cultures. The fermented roots were sun-dried separately and Lafun processed as described by Padonou *et al.* (2009a).

Sensory evaluation

Stiff porridges (Oka) were prepared using the Lafuns processed following the traditional method described by Padonou *et al.* (2009a) with use separately of 2Y48P22, 2Y48P22 + 2B24P31, 2Y48P22 + 1L48P35 and 2Y48P22 + 2L48P21 as starter cultures for the fermentations. Oka from a spontaneously fermented Lafun was also prepared. The stiff porridges were submitted to sensory analysis with a panel of 30 ordinary consumers using a triangular test (Meilgaard *et al.* 1999). The comparison was based on the flavour, colour and extensibility (the ability to stretch when a pinch of Oka was taken) of Oka. A hedonic test was performed on the Oka samples which were rated on a three-point scale (like, indifferent and dislike).

Enzyme assays

The ability of the yeast strain 2Y48P22, the LAB strains 1L48P35 & 2L48P21 and the *B. cereus* strain 2B24P31 to produce amylase and cell wall–degrading enzymes such as cellulase, polygalacturonase and pectinase was assessed.

Amylase activity was tested following the approach described by Oyewole (2001) that consisted in flooding during 2–5 min with iodine solution (Merck) 72 h cultures grown on Potato Dextrose Agar plates (Difco) containing 2% (w/w) soluble starch (Sigma-Aldrich S9765, Sigma-Aldrich, St Louis, MO). Production of amylase is evidenced by a clear zone around the colonies, while the rest of the plate stains blue-black.

The top agar method (Amoa-Awua and Jakobsen 1995) was used to test cellulase activity. The top agar contained 1% (w/v) agarose (Sigma-Aldrich, USA) and 0·5% (w/v) Carboxymethyl cellulose (Sigma C5678) in sodium phosphate buffer (pH 6·0). Wells were made into the agar and 30 μl of 3-day-old culture broth inoculated in the wells. The plates were incubated at 30°C during 24 h, flooded during 10 min with 0·1% (w/v) Congo Red (Gurr 8800; Colnbrook, Berks, UK) dissolved in sodium phosphate buffer (pH 7) and destained twice with 1 mol l^{-1} NaCl for 10 min each. Production of cellulase is evidenced by clearing zones in the red background of the plates.

Polygalacturonase activities were tested following the approach described by Gainvors *et al.* (1994). The cultures were incubated on agar media (MYGP for the yeast, MRS for the LAB and Nutrient agar for *B. cereus*) containing 1% (w/w) polygalacturonic acid (Sigma P3889) during 3–4 days at 30°C. They were stained for 5 min with 0·1% (w/v) Ruthenium Red (Sigma-Aldrich R275-1) and washed with sterile milli-Q water. Occurrence of polygalacturonate degradation is shown by a deep purple halo surrounding the colonies on the plates.

A similar approach was used to test pectinase activity. The agar plates were made up of 1% (w/v) pectin (Sigma P9135) and 1% (w/v) agarose (Sigma-Aldrich) dissolved in phosphate buffer (pH 6·0). Three-day-old cultures were inoculated in preformed wells and incubated at 30°C for 24 h. The plates were stained with 0·1% Ruthenium Red (Sigma-Aldrich) and washed twice with sterile milli-Q water. Pectinase activity is indicated by a red halo around the wells.

Statistical analysis

Data were analysed with the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Comparison of pH and stress at fracture σ_f from single culture fermentations was performed using either one-way ANOVA followed by Dunnett test and Student–Newman and Keuls for more than two microbial strains within a species group or Student's *t*-test for two strains. One-way ANOVA was performed to compare pH, stress at fracture and viable count from mixed culture fermentations.

Results

Screening by fermentations with single cultures and selection of strains for mixed culture fermentation and Lafun processing

Macroscopic examination and microscopic observation of cells from colonies grown on agar plates streaked by liquid from 48-h fermenting cassava flasks showed that the microbial population of the fermenting cultures was dominated by the inoculated micro-organisms (results not shown).

The pH and the stress at fracture of the fermented cassava pieces after 48 h fermentation time are presented in Table 1. Low stress value at fracture indicates softening of the cassava root. Fermented cassava from flasks inoculated by the yeast strains gave pH values of 5·62–5·75 at the end of the fermentation process. Two strains of *S. cerevisiae* (2Y48P22 and 1Y48P31) showed higher activity in the softening of the root, 2Y48P22 giving the softest cassava root pieces (Table 1). The *S. cerevisiae* 2Y48P22 was chosen for further experiments.

When the submerged cassava root was inoculated with LAB, the pH decreased from 6·28 to 6·37 at 0 h of fermentation to 3·92–4·56 at 48 h of fermentation (Table 1). Cassava inoculated with *Lact. plantarum* 1L48P35, 2L48P11, 2L0P14 and 2L0P31 showed no significant difference and gave the lowest pH at the end of the process. Among the *Lact. fermentum* strains screened, the lowest pH of the fermenting cassava (at probability level $P = 5\%$) was obtained with the flask inoculated by 2L48P21. The stress at fracture of cassava pieces fermented with all the *Lact. fermentum* strains was similar (Table 1). Based on these indices, the *Lact. plantarum* 1L48P35 and the *Lact. fermentum* 2L48P21 were chosen for the fermentation trials with mixed starter cultures.

None of the *B. cereus* stains showed significant differences either in the pH or in the softening of the root (Table 1). Because the five *B. cereus* strains tested behaved similarly regarding the pH of the fermented cassava and its stress at fracture, *B. cereus* 2B24P31 was selected randomly for further experiments.

Figure 1 shows the compression profiles of the fermented cassava recorded by the Instron uniaxial compression instrument. Cassava pieces fermented by 1L48P35, 2L48P21 and 2B24P31 displayed similar profile with pieces from the control flask giving the slope values ρ of their curves at levels of $3{\cdot}2 \times 10^6$–$4{\cdot}0 \times 10^6$ with $0{\cdot}2 \le \varepsilon < 0{\cdot}4$, indicating similar index of firmness (Steffe 1996), while the slope value ρ' of the 2Y48P22 curve was 5–6 times lower ($\rho' = 6{\cdot}4 \times 10^5$) (data not shown).

Mixed culture fermentation

Effect of the mixed starter cultures on pH and softening of the soaked cassava

The selected micro-organisms (2Y48P22, 1L48P35, 2L48P21 and 2B24P31) were combined in different ways and used as inocula for the mixed fermentation trials. Acidic pH ranging from 3·8 to 4·2 was recorded when the inoculum used contained the LAB 1L48P35 and/or 2L48P21, while slight decrease in pH was observed when the soaked cassava was inoculated by the combination 2B24P31 + 2Y48P22 (Table 2). This combination gave the softest product, with softness similar to the roots inoculated with the *S. cerevisiae* 2Y48P22 (Fig. 1). For the other combinations, inocula made up of the association of 2Y48P22 with any other LAB and/or 2B24P31 tend to soften the cassava pieces (Table 2).

Growth of the selected micro-organisms during the fermentation

Enumerations showed that when using the mixed starter cultures for fermentation, the yeast 2Y48P22 and the

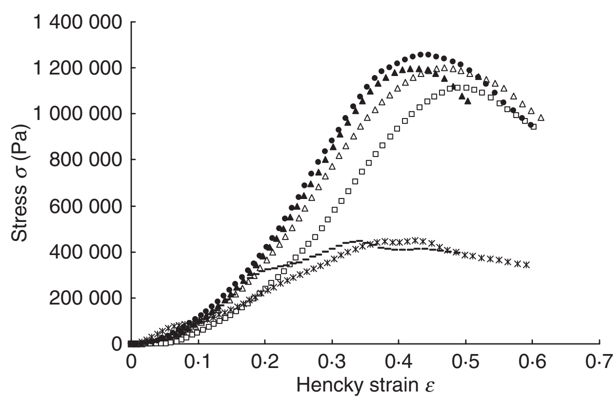

Figure 1 Compression profiles of cassava pieces fermented by the selected starter cultures expressed as a function of the stress σ applied on the cassava pieces during the compression and the intensity of the deformation because of the compression (Hencky strain ε). Profile of cassava pieces fermented by: (▲) *Lactobacillus p1antarum* 1L48P35, (Δ) *Bacillus cereus* 2B24P31, (□) *Lactobacillus fermentum* 2L48P21, (✱) *Saccharomyces cerevisiae* 2Y48P22, (—) *S. cerevisiae* 2Y48P22 + *B. cereus* 2B24P31, (●) control.

Table 2 pH and stress at fracture at 0 and 48 h fermentation time of the cassava fermented by mixed starter cultures of yeast, lactic acid bacteria and *Bacillus cereus**

Inoculated strains†	pH 0 h	pH 48 h	Stress at fracture σ_f (MPa) at 48 h fermentation time
2Y48P22 + 2L48P21 + 1L48P35 + 2B24P31	6.44 ± 0.07	4.11 ± 0.12[a]	0.89 ± 0.10[b]
2Y48P22 + 2L48P21 + 2B24P31	6.47 ± 0.18	4.11 ± 0.23[a]	0.92 ± 0.11[b]
2Y48P22 + 1L48P35 + 2B24P31	6.39 ± 0.10	4.21 ± 0.07[a]	0.83 ± 0.15[b]
2L48P21 + 2B24P31	6.40 ± 0.21	3.84 ± 0.22[a]	1.02 ± 0.10[bc]
1L48P35 + 2B24P31	6.35 ± 0.21	3.77 ± 0.24[a]	1.08 ± 0.13[bc]
2Y48P22 + 2L48P21	6.46 ± 0.16	4.19 ± 0.11[a]	0.93 ± 0.10[b]
2Y48P22 + 1L48P35	6.51 ± 0.06	4.24 ± 0.09[a]	0.97 ± 0.05[b]
2Y48P22 + 2B24P31	6.36 ± 0.18	5.34 ± 0.22[b]	0.59 ± 0.16[a]
Non inoculated fermentation	6.42 ± 0.08	5.29 ± 0.26[b]	1.18 ± 0.09[c]

*Results given as mean of data from three fermentations ± SD. Data with different superscript letters in a column are significantly different ($P < 5\%$).
†For explanation see Table 1.

LAB 1L48P35 and 2L48P21 grew throughout the process reaching levels of 7 $\log_{10}$ and 9 $\log_{10}$ CFU g^{-1}, respectively, while *B. cereus* 2B24P31 failed to grow (Table 3). Similar results were observed during Lafun spontaneous fermentations (Padonou *et al.* 2009b). Although bacterial growth was recorded in the control flask, LAB and *Bacillus* spp. counts were reduced at levels of 1–5 log-units compared with the inoculated flasks, showing that the inoculated organisms were predominant during the fermentations.

Use of starter cultures for Lafun processing

The stiff porridges Oka obtained from Lafun fermented by the inoculation process were judged different from the one coming from the spontaneously fermented Lafun regarding the response of panellists on the colour (92% of the panellists), the extensibility (86% of the panellists) and the flavour (72% of the panellists). Lafun fermented using *S. cerevisiae* 2Y48P22 gave the whitest and most extensible Oka with a desirable flavour preferred by 100% of the panellists (Table 4).

Enzymatic activities of the selected starter cultures

The strains were checked for amylase, cellulase, pectinase and polygalacturonase activities. While the *Lact. plantarum* 1L48P35, the *Lact. fermentum* 2L48P21 and the *B. cereus* 2B24P31 showed negative reaction for all these tests, the yeast *S. cerevisiae* 2Y48P22 was positive for pectinase production (Fig. 2) and negative for the cellulase and polygalacturonase tests.

Table 3 Viable counts at 0 and 48 h fermentation time of the cassava fermented by mixed starter cultures of yeast, lactic acid bacteria (LAB) and *Bacillus cereus**

Inoculated strains†	Viable counts ($\log_{10}$ CFU g^{-1}) Yeasts 0 h	Yeasts 48 h	LAB 0 h	LAB 48 h	*Bacillus* spp. 0 h	*Bacillus* spp. 48 h
2Y48P22 + 2L48P21 + 1L48P35 + 2B24P31	2·7 ± 0·7[a]	7·2 ± 0·0[a]	4·9 ± 0·4[a]	8·6 ± 0·1[a]	5·8 ± 0·6[a]	3·9 ± 0·5
2Y48P22 + 2L48P21 + 2B24P31	3·3 ± 0·2[a]	7·2 ± 0·1[a]	5·2 ± 0·2[a]	8·6 ± 0·6[a]	5·9 ± 0·3[a]	3·9 ± 0·2
2Y48P22 + 1L48P35 + 2B24P31	3·6 ± 0·1[a]	7·2 ± 0·3[a]	4·7 ± 0·4[a]	8·7 ± 0·2[a]	6·5 ± 0·3[a]	4·3 ± 0·2
2L48P21 + 2B24P31	<2	<2	4·8 ± 0·6[a]	8·7 ± 0·2[a]	6·3 ± 0·3[a]	4·2 ± 0·1
1L48P35 + 2B24P31	<2	<2	5·0 ± 0·1[a]	8·9 ± 0·2[a]	6·5 ± 0·4[a]	3·8 ± 0·4
2Y48P22 + 2L48P21	2·9 ± 0·4[a]	7·4 ± 0·6[a]	5·2 ± 0·3[a]	8·6 ± 0·4[a]	<2	<2
2Y48P22 + 1L48P35	3·2 ± 0·1[a]	7·4 ± 0·2[a]	5·1 ± 0·5[a]	8·5 ± 0·1[a]	<2	<2
2Y48P22 + 2B24P31	3·2 ± 0·3[a]	7·3 ± 0·2[a]	<2	4·5 ± 0·3[b]	6·4 ± 0·2[a]	4·2 ± 0·7
Noninoculated fermentation	<2	<2	<2	3·9 ± 0·7[b]	<2	3·2 ± 0·3

*Results given as mean of data from three fermentations ± SD. Data with different superscript letters in a column are significantly different ($P < 5\%$).
†For explanation see Table 1.

Table 4 Panellists overall preference of Oka made from Lafun fermented by different starter cultures of yeast, lactic acid bacteria and *Bacillus cereus*

Inoculum used in Lafun fermentation*	Opinion of panellists (% of 30 panellists)		
	Like	Indifferent	Dislike
NF†	35	46	19
2Y48P22	100	0	0
2Y48P22 + 2B24P31	61	35	4
2Y48P22 + 1L48P35	0	23	77
2Y48P22 + 2L48P21	0	0	100

*For explanation see Table 1.
†Spontaneous fermentation.

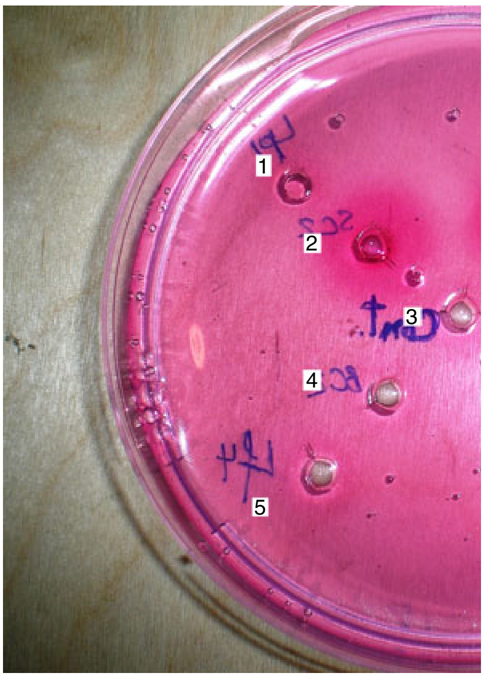

Figure 2 Pectinase activity assays. A red halo around the well 2 inoculated by *Saccharomyces cerevisiae* 2Y48P22 indicates a pectinase activity. The other wells were inoculated by: 1 = *Lactobacillus p1antarum* 1L48P35; 3 = sterile deionized water (control); 4 = *Bacillus cereus* 2B24P31; and 5 = *Lactobacillus fermentum* 2L48P21.

Discussion

Spontaneous cassava fermentations for Lafun production are usually associated with the softening of the submerged root (Oyewole and Odunfa 1988; Padonou *et al.* 2009b). This phenomenon has been observed in other cassava-based fermentations such as Foo-Foo (Okafor *et al.* 1984; Ampe and Brauman 1995; Brauman *et al.* 1996) and Agbelima (Amoa-Awua *et al.* 1996). Different groups of micro-organisms have been assumed to contribute to the softening of the root. Previously, *Citrobacter freundii* has been reported to be responsible for the softening of cassava roots (Okolie and Ugochukwu 1988). Lund (1972) and Brauman *et al.* (1995) described some pectolytic clostridia responsible for root crops softening. According to Juven *et al.* (1985) and Sakellaris *et al.* (1989), *Lact. plantarum* and *Leuconostoc mesenteroides* might contribute to cassava root softening as they were shown to produce cell wall–degrading enzymes such as polygalacturonases and polygalacturonate lyases. A *Corynebacterium* sp. has also been reported to produce a pectinolytic enzyme (Okafor *et al.* 1984). The microbial species most often reported as involved in cassava root softening are *Bacillus* spp. (Okafor *et al.* 1984; Amoa-Awua and Jakobsen 1995; Salvador *et al.* 2002; Mante *et al.* 2003; Coulin *et al.* 2006). Amoa-Awua and Jakobsen (1995) reported that *Bacillus* spp. dominated the microflora during Agbelima fermentation reaching levels of 10^7–10^8 CFU g^{-1} and being responsible for the breakdown of cassava texture through cellulase activity. However, in this study, *Bacillus* spp. numbers decreased throughout the fermentation process as previously observed by Oyewole and Odunfa (1988). Furthermore, the *B. cereus* strains used in this study did not induce any softening of the cassava root, probably because of the inability to grow under the submerged fermentation conditions. The high acidity of the fermenting medium (Oyewole and Odunfa 1988) and the low oxygen tension of the steeping water (Oyewole 1990) may also explain the inability of *Bacillus* spp. to grow well on the cassava roots in the submerged lactic fermentation conditions, while they are less inhibited under more aerobic conditions like Agbelima or Akyeke fermentations (Amoa-Awua and Jakobsen 1995; Obilie *et al.* 2003). Several filamentous fungi and yeast, among these *Candida krusei, Candida tropicalis, Pichia saitoi, Pichia anomala, Saccharomyces cerevisiae, Zygosaccharomyces florentinus* and *Zygosaccharomyces* spp., have also been reported to contribute to cassava tissue degradation (Amoa-Awua *et al.* 1997; Oyewole 2001; Jespersen 2003; Obilie *et al.* 2003; Dzogbefia *et al.* 2008). The involvement of the aforementioned yeast species especially pectinolytic *S. cerevisiae* in cassava root softening during fermentations appears not to be demonstrated earlier. However, pectin-degrading enzyme production has been reported for *S. cerevisiae* from other sources (McKay 1990; Gainvors *et al.* 1994; Blanco *et al.* 1998; Hirose *et al.* 1998; Gognies *et al.* 1999). The *S. cerevisiae* pectinolytic enzyme reported is an endo-polygalacturonase encoded by a gene *PGU1* (Blanco *et al.* 1998) also named *PSM1* (Hirose *et al.* 1998) or *PGL1* (Gognies *et al.* 1999). The present work showed evidence that the predominant strain of *S. cerevisiae* in Lafun contributed to cassava root softening during the submerged fermentation process. It also proved the possibility to obtain good-quality Lafun by the use of *S. cerevisiae* as starter culture for cassava fermentation. However, when *S. cerevisiae* 2Y48P22 is used singly as starter culture, the risk of survival of pathogens may exist because the final pH of the fermented product was about 5–6. The combined use of *S. cerevisiae* 2Y48P22 with the LAB as starter cultures should be considered for safe and reproducible production of Lafun.

Conclusion

This work confirmed that souring of submerged cassava root is mostly because of LAB activities. *S. cerevisiae* 2Y48P22 was showed to be of great importance for softening of the roots during the fermentation. The role of *B. cereus* was not clearly evidenced in this study; it is likely that *Bacillus* spp. does not play a significant role in Lafun production. Whereas LAB and yeasts grew throughout the process, *B. cereus* decreased in numbers and did not contribute to softening of the roots, possibly because of low oxygen availability and high acidity under the submerged conditions. *S. cerevisiae* 2Y48P22 was demonstrated to be a suitable starter culture for Lafun. For further studies, attention should be focused on the combined use of this strain of *S. cerevisiae* with LAB to ensure a lower pH and improve food safety. The cassava softening activity of S. cerevisiae 2Y48P22 appears to be induced by pectin-degrading enzymes. Further studies will investigate the enzymes produced by the *S. cerevisiae* 2Y48P22 responsible for cassava softening during Lafun processing.

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