UNIVERSITE D'ABOMEY-CALAVI

FACULTE DES SCIENCES AGRONOMIQUES

Preserving African Food Microorganisms for Green Growth

(Green Growth for Africa)

The use of starter cultures from the UAC culture collection to ferment cassava for lafun production by Alitech Industries, a medium scale food processing company in Benin

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1. INTRODUCTION

Selected small and medium enterprises (SMEs) involved in fermented food processing in Benin were trained under GreenGrowth project for business model development and for the use of starter cultures to improve their activities. Alitech Industries Sarl is a medium scale food processing enterprise trained in this frame. This company has a partnership with a French company named “Racines” for the exportation of cassava derived products, including *Lafun*, a fermented cassava flour. Alitech Industries is facing a problem of contamination of its *Lafun* by *Bacillus cereus* at non acceptable levels, i.e beyond 10^3 germs/g. As previous studies demonstrated that the microbiota associated with *Lafun* fermentation is predominated by lactic acid bacteria (LAB) and yeasts (Padonou et al., 2009), this was for UAC an opportunity to experiment with Alitech the use of isolated LAB and yeasts cultures to ferment cassava for *Lafun* production with safe and reliable quality. Thus, before being put at Alitech disposal a stable form of starter to better its fermentations, LAB and yeast isolated from *mawè* fermentation under GreenGrowth project (Houngbédji et al., 2018) as well the yeast 2Y48P22 isolated from cassava fermentation under ENRECA project (Padonou et al., 2009), all showing desirable technical properties and preserved in the UAC biobank, were experimented in the company for *Lafun* production with less contamination by *B. cereus*.

2. Starter cultures preparation: microbial species included in the experiment and inocula preparation

Two LAB species (*Lactobacillus plantarum* LP1 and *Lactobacillus fermentum* LF2) both isolated from *mawè* fermentation, and two yeasts species (*Kluyveromyces marxianus* Km3 from *mawè* fermentation, and *Saccharomyces cerevisiae* Scp from *Lafun* fermentation) were involved in the experiment (Table 1). Inocula were prepared in the Laboratory of Food Sciences of UAC following the procedure described by Padonou et al. (2010). Four mixed cultures were obtained:

- 1 inoculum combining *Lactobacillus fermentum* and *Saccharomyces cerevisiae*, (Lf2+ScP)
- 1 inoculum made up of *Lactobacillus fermentum* and *Kluyveromyces marxianus*, (Lf2+Km3)
- 1 inoculum composed by *Lactobacillus plantarum* and *Saccharomyces cerevisiae* (Lp1+ScP)
- 1 inoculum consisting in *Lactobacillus plantarum* and *Kluyveromyces marxianus* (Lf2+Km3)

The inocula were transported aseptically on ice to Alitech Industries site (20 - 30 minutes). Each mixed culture was used as starter culture to ferment cassava. The cultures were inoculated to reach high levels of 10^7 cells/mL for LAB and 10^5 cells/mL for yeasts in small scale fermenting batches (5 kg of peeled cassava chunks fermented in 6 litres of water). The high levels inoculated cells were expected to compete with endogenous microflora, particularly *B. cereus* from the onset of the fermentation. The best composite starter culture in terms of speed of fermentation (decrease of pH and acid formation), cassava softening, *B. cereus* inhibition during the fermentation and in the final product will be chosen and put at Alitech disposal for further fermentations. The *Lafun* that will be obtained is expected to harbour less than 10^2 germs of *Bacillus cereus*/g of *Lafun*, a required level by customers.

Table 1. Microbial strains used as starter cultures in the experiment.

Nr	Microbial species	code	UAC biobank accession nr	Genbank accession nr
1	*Lactobacillus plantarum*	LP1	UAC-01-338-1	MG 245815
2	*Lacotbacillus fermentum*	LF2	UAC-01-13-1	MG 245804
3	*Kluyveromyces marxianus*	Km3	UAC-01-381-1	MG 245826
4	*Saccharomyces cerevisiae*	Scp	UAC-01-379-1	EU 439442

Experimental plan

Inocula prepared in the laboratory

Inoculation

3. Preliminary results

48 hours fermentation time were observed for all batches. During this time:

- LAB counts increased from 10^7 CFU/mL (0 h) to 10^9 CFU/mL (48 h);
- Yeasts counts increased from 10^5 CFU/mL to 10^6 CFU/mL;
- *Bacillus cereus* counts decreased from 10^6 CFU/mL and were not detectable at the end of the fermentation (< 10 CFU/mL);
- pH of the fermenting batches decreased from 5.4-6.5 to 4.1-4.2;
- Titratable acidity increased from 3.0%-3.6% to 0.95%-1.10%

The end products obtained after squeezing and drying the fermented cassava were analysed for *B. cereus* contamination. Results showed that all the *Lafun* obtained had less than 10 CFU/mL of *B. cereus*. This results show that *B. cereus* were successfully inhibited during the

fermentation and the good processing practices applied for squeezing and drying allowed the production of safer and microbiologically acceptable Lafun.

pH, titratable acidity and softening of fermented cassava were compared to choose the best inoculum for larger scale fermentations. The inoculum Lp1+Km3 gave the best result. However, as it was observed that *Kluyveromyces marxianus* is most sensitive while *Saccharomyces cerevisiae* is resistant under stress conditions during fermentations (Houngbédji et al, unpublished), it was suggested to combine both yeast species with the LAB Lp1 to obtain a three-strains composite inoculum (Lp1+Km3+Scp).

4. Work forward

To deeper the trial carried out with Alitech, the inoculum Lp1+Km3+Scp was used to ferment at larger scale a cassava batch (1 ton of cassava) for Lafun production. In parallel, two other non-inoculated batches were fermented, one under researchers' control, and the second according to the procedure usually followed by Alitech. All fermentations were followed up, sampled. Microbiological analyses are currently on progress and results will be compared to evidence the advantages of the use of starter cultures for Lafun processing.

5. CONCLUSION

The transfer and the use of starter cultures at SMEs scale is a goal to be achieved through the GreenGrowth project to help fermented food processing companies to provide consumers with safe and nutritious foods. In Benin, Alitech Industries already encounters problem of safety of its Lafun which is highly contaminated by *B. cereus*. The use of appropriate starter culture could allow to obtain a safer Lafun for human consumption.

6. References

Houngbédji M. Johansen P., Padonou S.W., Akissoé N., Arneborg N., Nielsen D.S., Hounhouigan D.J., Jespersen L. (2018). Occurrence of lactic acid bacteria and yeasts at species and strain level during spontaneous fermentation of mawè, a cereal dough produced in West Africa. *Food Microbiology* 76, 267–278.

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Padonou S.W., Nielsen D.S., Akissoe N.H., Hounhouigan J.D., Nago M.C. et Jakobsen M. (2010).Development of starter culture for improved processing of Lafun, an African fermented cassava food product. *Journal of Applied Microbiology* 109, 1402–1410.

Appendices

Table 1: pH and titratable acidity of the fermenting medium

Inoculum	Sampling time	pH	Titratable acidity
Control	0h	6.5 ± 0.0	0.30 ± 0,05
	6h	5.4 ± 0.2	0.35 ± 0,01
	24h	4.2 ± 0.2	0.95 ± 0,23
	48h	4.2 ± 0.2	1.10 ± 0,36
LP1SCP	0h	6.0 ± 0.1	0.36 ± 0,04
	6h	4.9 ± 0.0	0.38 ± 0,02
	24h	4.4 ± 0.1	0.86 ± 0,09
	48h	4.2 ± 0.4	0.97 ± 0,35
LP1KM3	0h	5.5 ± 0.8	0.33 ± 0.01
	6h	4.8 ± 0.2	0.37 ± 0.03
	24h	4.2 ± 0.1	0.87 ± 0.36
	48h	4.1 ± 0.3	0.97 ± 0.31
LF2SCP	0h	5.6 ± 0.3	0.34 ± 0.01
	6h	4.8 ± 0.0	0.39 ± 0.07
	24h	4.4 ± 0.1	0.83 ± 0.06
	48h	4.2 ± 0.3	0.95 ± 0.24
LF2KM3	0h	5.4 ± 0.4	0.35 ± 0.00
	6h	4.7 ± 0.1	0.42 ± 0.03
	24h	4.2 ± 0.1	0.92 ± 0.27
	48h	4.1 ± 0.3	1.01 ± 0.29

Table 2: LAB counts (log10 CFU/ml) in the fermenting medium.

Sampling time (h)	Inoculum type			
	LF2SCP	**LF2KM3**	**LP1SCP**	**LP1KM3**
0	7.2	7.2	7.95	7.95
6	7.95	8.3	8.7	8.6
24	8.9	8.7	8.6	8.8
48	8.8	8.9	9.1	8.7

Table 3: Yeast counts (log10 CFU/ml) in the fermenting medium.

Sampling time (h)	Inoculum type			
	LF2SCP	**LF2KM3**	**LP1SCP**	**LP1KM3**
0	4.2	4.5	4,25	4.8
6	4.3	4.7	5	5
24	5.3	4.8	5	5.6
48	5.8	4.9	5,85	5.3

Table 4: Bacillus cereus counts (log10 CFU/ml) in the fermenting medium.

Sampling time (h)	Inoculum type			
	LF2SCP	**LF2KM3**	**LP1SCP**	**LP1KM3**
0	6	5.9	6.1	5.5
6	6.1	6.2	6.4	6.4
24	ND	3.6	ND	ND
48	ND	ND	ND	2