

BIODIVERSITY OF
LACTIC BACTERIA
DURING THE
FERMENTATION OF
*NTOBA MBODI (Fermented
cassava leaves)*

INTRODUCTION

The *ntoba mbodi* obtained by fermentation of cassava leaves (*Manihot esculenta Crantz*) is a fermented vegetable which is consumed in Congo.

The fermentation of cassava leaves is performed by traditional way using several processes. These processes allow, not only, to eliminate the toxic cyanogenic compounds in the cassava leaves, but also to raise the flavour of the fermented product.

The pH of *ntoba mbodi* increases during the fermentation and becomes alkaline at the end (pH8.5-8.9).

Lactic bacteria were found to be dominant at the beginning, whereas *Bacillus* were found to be the major at the end of the fermentation of *ntoba mbodi*.

The lactic acid bacteria thus remain present throughout the alkaline fermentation of the cassava leaves.

The main objective of this work was to confirm the presence of the lactic bacteria in the alkaline product and to check for their biodiversity in *ntoba mbodi* in perspective for a production by controlled fermentation.

Production of *ntoba mbodi*



1

Cassava leaves



2

Cassava leaves slightly dried and cut



3

Peaces of leaves in big leaves of bilordia and/or papaya for wrapping



4

Wrapped leaves for fermentation

5



Fermented leaves: *ntoba mbodi*

Identification of lactic acid bacteria

53 bacteria were isolated from different production sites of Congo and identified by phenotyping (including API 50CHL) and genotyping (ITS-PCR, Rep-PCR and DNA sequencing)

RESULTS

The lactic bacteria are divided into 4
genera: *Weissella*,
Enterococcus,
Lactobacillus
and *Pediococcus*.

The species are

Weissella confusa (45.3%)

Weissella cibaria (7.6%),

Enterococcus casseliflavus (16.9%),

Enterococcus faecium (5.6%),

Enterococcus hirae (3.7%),

Enterococcus faecalis (3.7%)

Enterococcus avium (1.8%),

Lactobacillus plantarum (9.4%)

Pediococcus pentosaceus (5.6%).

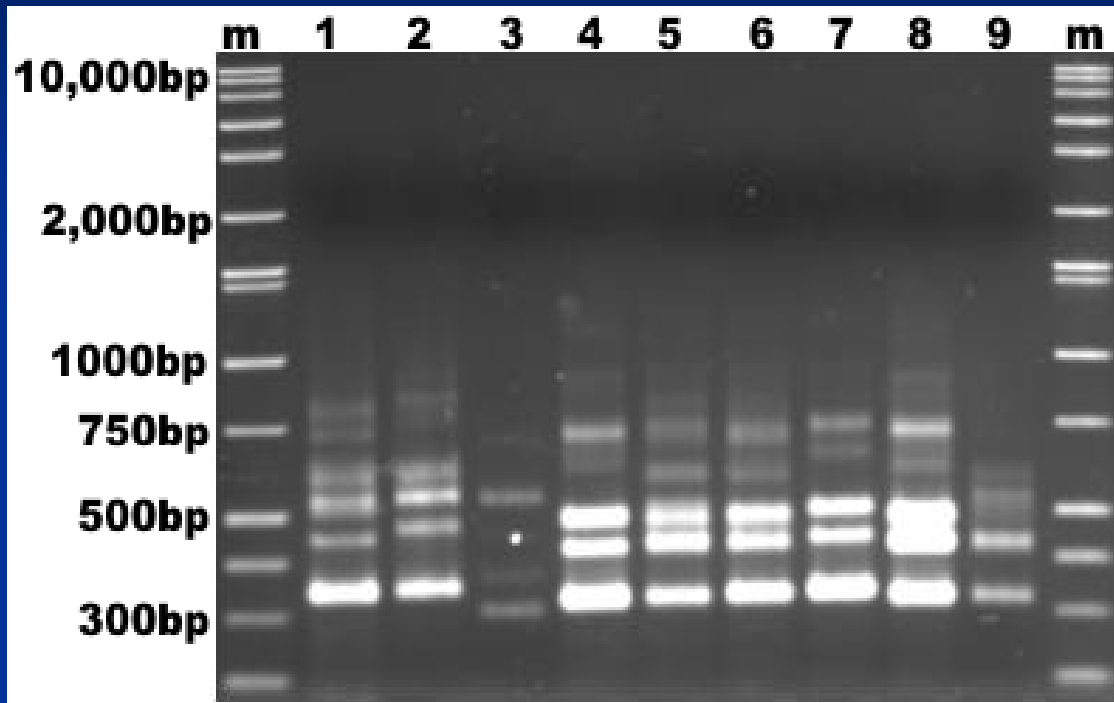
Distribution of the lactic bacteria populations during the fermentation of the cassava leaves

The initial lactic microflora includes:
Weissella (88.88%), of which *Weissella confusa* (77.77%) and *Weissella cibaria* (11.11%) and *Enterococcus* (11.11%)

After 24 hours of fermentation:
Weissella constitutes the dominant microflora (90%). It is supplanted at 48 hours by *Enterococcus* (41.17%) and *Lactobacillus* (29.41%).

At the end of the fermentation (72 hours):

the lactic acid microflora includes the different kind of *Weissella* (50%), *Enterococcus* (42.85%) and *Pediococcus* (7.14%).



m: standard marker

1: *W. confusa*

2: *W. cibaria*

3: *Lb. Plantarum*

4: *Ec. casseliflavus*

5: *Ec. faecium*

6: *Ec. hirae*

7: *Ec. avium*

8: *Ec. faecalis*

9: *Pc. Pentosaceus*

Figure 1: Electrophoretic ITS-PCR profiles of group of the isolated lactic acid bacteria during the fermentation of the cassava leaves in *ntoba mbodi*

- 1: *W. confusa* 1.1
- 2: *W. confusa* 1.2
- 3: *W. confusa* 1.3
- 4: *W. confusa* 1.4
- 5: *W. confusa* 1.7
- 6: *W. confusa* 1.5
- 7: *W. confusa* 1.6
- 8: *W. cibaria* 2.1
- 9: *W. cibaria* 2.2
- 10: *Lb. plantarum* 3.1

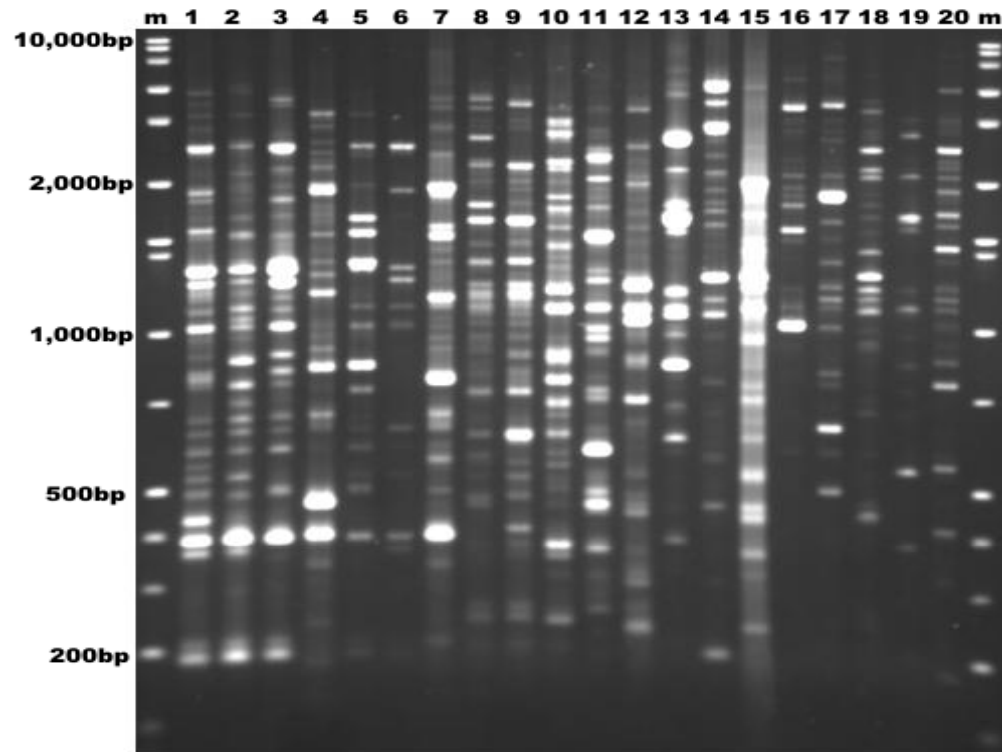


Figure 2: Profiles of under species of the lactic bacteria isolates obtained by electrophoresis of the Rep-PCR

The molecular methods of identification of the lactic bacteria allowed a confirmation of the importance of lactic acid bacteria involved in alkaline fermentation, a revelation of the large diversity of the lactic acid bacteria in *ntoba mbodi* and an identification of bacteria which may be utilised for the improvement of the production of *ntoba mbodi*.

DISCUSSION

W. confusa is dominant lactic acid bacteria in *ntoba mbodi*.

The high frequency suggests that *W. confusa* may takes an active part in the fermentation process.

Lactobacillus plantarum is in small quantity (9.4%), it is the case of *Pediococcus*.

Pediococcus are interesting for the production of pediocin A which is an atypical bacteriocin, thermotolerant and nonproteinic.

The *Enterococcus* are lactic bacteria which are important in environmental, food and clinical microbiology.

5.66% of the lactic bacteria strains (3.77% for *Enterococcus faecalis* and 1.8% for *Pediococcus pentosaceus*) isolated and identified during the fermentation of *ntoba mbodi* have a pectinolytic activity. These strains are also involved in the softening of the cassava leaves.

Several strains of *Lactobacillus* (*Lb. casei*, *Lb. paracasei subsp. tolerans*, *Lb. curvatus*, *Lb. pentosus* and *Lb. plantarum*) isolated from the vegetable material present a strong tolerance to the alkaline pH, with pH maxima located between 8.5 and 8.9

CONCLUSION

The alkaline fermentation of cassava leaves in *ntoba mbodi* involves various genera and specie of lactic acid bacteria.

The lactic bacteria have a very great diversity of metabolic capacities: growth at various temperatures, tolerance with acid or basic pH.

Thank you
for your attention

Model of potassium extrusion at alkaline pH.

Mechanisms involved ,A TP-linked K^+/H^+ antiport-system; passive diffusion of membrane-permeable weak base (Am as unprotonated amine and AmH^+ as protonated amine); the nature of this K^+ pathway is unknown; sodium ATPase which exchanges Na^+ for K^+ . (KAKINUMA Y. et al., 1988)

