

Research Article

Towards the Understanding of Fermented Food Biotechnology in Congo Brazzaville

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Abstract: Congo Brazzaville harbor a diversity of traditional fermented foods which involve complex interactions between various microorganisms groups and different molecules in detoxification, texture and organoleptic features. This first retrospective review aims to describe the various works that have been done regarding the biochemical and microbiological features of our traditional fermented foods including Nsamba, Poto-poto, Bikedi and Ntoba-Mbodi.

Keywords: Bacteriocin, Congo Brazzaville, diversity, lysinibacilluslouembei, probiotic, traditional fermented food

INTRODUCTION

Fermented foods play an important role in the daily diet in Congo Brazzaville. Cereals, fruits, vegetables, tubers and fish are various raw materials used in fermented foods. Among fermented food, *Pilipili*, *Bikedi*, *foufou*, *Chikwangue*, *Poto-poto* and *Ntoba Mbodi* are the most representatives (Louémbé *et al.*, 1996, Louémbé *et al.*, 2003b; Miambi *et al.*, 2003). Other fermented food including *Tutu*, *Tsiya* and fermented fish can be found (Table 1). The traditional fermented beverages are produced according to the areas of raw materials. Among beverages *Loungouila*, *Bounganda* and *Nsamba* are the most experienced (Louémbé *et al.*, 1986; Malonga *et al.*, 1995). Others beverages like *Malala*, pineapple wine, orange wine and *Mbamvou* are also found in Congo (Table 1). Fermented foods and beverages involve metabolic activities of microorganism and/or consortia (Louémbé *et al.*, 2003b; Malonga *et al.*, 1993; Ouoba *et al.*, 2010).

Technological processes for the traditional food production vary between areas (Table 2). However, they are generally based on the same techniques (Louémbé *et al.*, 1986, 1996; Louémbé *et al.*, 2003b; Malonga *et al.*, 1995). It is done with a non-sterile equipment and non-optimized conditions, resulting in food with an unpredictable nutritional quality (Olanrewaju *et al.*, 2009).

Basing on microorganism diversities, enzymes, organoleptic molecules and preservative properties, this first review aims to describe four Congo traditional fermented foods including *Nsamba* (NS), *Poto-poto* (PP), *Bikedi* (Bk) and *Ntobambodi* (NM).

Palm wine "NSAMBA (NS)": NS is the most consumed in Congo Brazzaville and more representatives among traditional fermented drink. It is obtained from a natural fermentation of the sweet sap of the oil palm (*Elaeisguineensis*). NS composition is about 1.5 to 2.1% alcohol (Bassir, 1962), pH between 4-5 (Faparusi and Bassir, 1972), 12 and 15% of soluble sugar (sucrose). There are also small amounts of glucose, fructose, raffinose, maltose, polysaccharides, organic acids and amino acids (Van Pee and Swings, 1971). NS Micro organisms differentiate from urban to rural areas. Lactic acid bacteria (LAB), acetic bacteria, *Zymomonas* spp., Micrococci and yeasts are more found (Faparusi, 1973; Okafor, 1972a, 1972b, 1975a, 1975b; Malonga *et al.*, 1995). Yeasts, especially *Saccharomyces cerevisiae*, are less predominant than the homolactic and heterolactic bacteria. The consortia of heterolactic bacteria and yeast lead alcohol production from the palm sap to generate NS (Malonga *et al.*, 1995; Louémbé *et al.*, 1986). *Bacillus subtilis*, *B. sphaericus*, *B. pumilus*, *Corynebacterium* spp., *Lactococcus lactis*,

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Table 1: Non-exhaustive list of various fermented foods and beverages met in Congo Brazzaville

Common Name	Area	Raw material	Characteristics	References
Fermented foods				
Ntoba Mbodi or Kingouari	Bouenza PoolBrazzaville Pointe Noire	<i>Manihotesculenta</i> Crantz (leaves)	Fermented cassava leaves (3 to 4 days)	Louembé <i>et al.</i> (2003b) and Ouoba <i>et al.</i> (2010)
Bikédi	National food	<i>Manihotesculenta</i> Crantz (tubers)	Fermented cassava tubers (2 to 4 days)	Malonga <i>et al.</i> (1993, 1996), Louembé <i>et al.</i> (1997) and Kobawila <i>et al.</i> (2005)
Foufou	National food	<i>Manihotesculenta</i> Crantz (tubers)	Flour fermented cassava tubers	Not studied
Chikwangué	National food	<i>Manihotesculenta</i> Crantz (tubers)	Fermented cassava dough	Miambi <i>et al.</i> (2003)
Mbalampinda	Bouenza Niari	<i>Manihotesculenta</i> Crantz (tubers)	Fermented cassava dough mixed with peanut paste	Not studied
Tutu	North of Country	<i>Manihotesculenta</i> Crantz (tubers)	Starched and gelled mass of cassava flour	Not studied
Tsaba	North of Country	<i>Manihotesculenta</i> Crantz (tubers)	Cassava tubers fermented	Designed and unpublished
Minsela	North of Country	<i>Landolphi ajumelei</i>	cooked lamellae	
Tsiya	North of Country	<i>Landolphi ajumelei</i>	Fermented pulp of a bay	Not studied
Poto-poto	National food	<i>Zea mays</i> (maize seeds)	<i>Landolphi ajumelei</i> fermented maize rough	Louembé <i>et al.</i> (1996, 2003a) and Louembé <i>et al.</i> (2004)
Pilipili or Pidipidi	National food	<i>Capsicum</i> spp.(Chili)	Fermented chili sauce	Designed and unpublished
Mpandé	North of Country	<i>Raphiafarinifera</i>	Pulp of the fermented raffia palm nuts	Not studied
Moussa or Ngayi-Ngayi	Pool	<i>Hibiscus sabdarifa</i>	Fermented fruit sorrel	Not studied
<i>Fermented beverages</i> Nsamba	National food	<i>Elaeisguineensis</i>	Palm wine	Malonga <i>et al.</i> (1995) and Louembé <i>et al.</i> (1986)
Bounganda or Loutoko or Biyoki	Bouenza Niari North of Country	<i>Zea mays</i> <i>Manihotesculenta</i> Crantz	Aqueous mixture of germinated maize flour and starched cassava flour	Not studied
Loungouila	Bouenza	<i>Saccharumofficinale</i>	Sugar cane wine obtained after fermentation of week	Outstanding Studies
Douma	North of country	Honey and wild water	Honey wine	Not studied
Ntombé	Lekoumou Niari	<i>Elaeisguineensis</i>	The fermented sap of the palm trunk	Not studied
Malala	Bouenza	<i>Cirus</i> sp.	Basing on grapefruit	Not studied
Ananas wine	Bouenza	<i>Ananas comosus</i> (fruits)	A fruit and pineapple juice	Not studied
Orange wine	Bouenza	<i>Citrus sinensis</i> (fruits)	Basing on orange fruit	Not studied
Mbamvou	Bouenza	<i>Musa</i> sp.	Fermented Bananas	Not studied

Table 2: Flowchart of production of three traditional fermented foods of Congo Brazzaville (Louembé *et al.*, 1996, 2003b; Malonga *et al.*, 1996)

A: Production of <i>Bikedi</i> (fermented cassava tubers)	B: Production of <i>Ntobambodi</i> (fermented cassava leaves)
Peeled Cassava tubers	Cassava leaves
↓	↓
Immersion in water for fermentation (3 to 4 days)	Partial dehydration with ambient air
↓	↓
Removing retted tubers from water	Cut into pieces
↓	↓
Washing and draining	Clean with water
↓	↓
Bikedi (Bk)	Packing in papaya leaves
	↓
	Fermentation (2 to 4 days)
	↓
	NtobaMbodi (NM)
C: Production of <i>Poto-poto</i> (fermented maize)	
Dried corn kernels	
↓	
Soaking in tap water	
↓	
Draining	
↓	
Grinding	

Table 2: Continue

↓
 wet flour
 ↓
 Defibrillation using water through a muslin cloth
 ↓
 Settling for 15 hours
 ↓
 Dripping with burlap
 ↓
Poto-poto (PP)

Table 3: Isolated microorganisms from NS

Raw material	Product	Microorganisms	References
Oil palm <i>Elaeisguineensis</i>	Nsamba (Palm wine)	Bacteria <i>Bacillus subtilis</i> <i>B. sphaericus</i> <i>B. pumilus</i> <i>Corynebacterium</i> spp. <i>Hanseniaspora guilliermondii</i> <i>Pseudomonas fluorescens</i> <i>Staphylococcus piscifermentans</i> <i>Staphylococcus</i> spp. <i>Streptococcus</i> spp. <i>Strept. Lactis</i> ssp. <i>Lactobacillus casei</i> <i>Lb. plantarum</i> <i>Ln. mesenteriodes</i> ssp <i>Ln. lactis</i> <i>Pediococcusparvulus</i> <i>Acetobacter pomorum</i> <i>Acetobacter pasteurianus</i> <i>Gluconobacter oxydans</i> <i>Acinetobacter calcoaceticus</i> <i>Enterobacterium bacterium</i> <i>Acidovorax</i> sp. <i>Comamonas</i> sp. Yeast <i>Saccharomyces cerevisiae</i>	Malonga <i>et al.</i> (1995) and Louémbé <i>et al.</i> (1986)

Streptococcus spp., *Hanseniaspora guilliermondii*, *Pseudomonas fluorescens* and *Staphylococcus* spp. were also found (Malonga *et al.*, 1995) (Table 3). The 16S rRNA gene analysis revealed 32 community clones were identified as *Lb. casei* strain zhang, *Lb. plantarum*, *Leuconostoc mesenteriodes*, *Ln. lactis*, *Pediococcus parvulus* strain Bpe-299, *Acetobacter pomorum*, *Acetobacter pasteurianus*, *Gluconobacter oxydans*, *Acinetobacter calcoaceticus*, *Enterobacterium bacterium*, *Acidovorax* sp., *Comamonas* sp., *B. subtilis*, *Staphylococcus piscifermentans* (Okolie *et al.*, 2013). This finding matched with previous studies assessed in palm wine in Congo Brazzaville (Louémbé *et al.*, 1986; Malonga *et al.*, 1995) (Table 3).

Poto-poto: fermented dough of maize: In Africa, the fermentation of maize (*Zea mays*) is achieved through an extremely long process. This dough is called *ujior akamu* in Kenya (Akingbala *et al.*, 1981), *ogiorakamu* in Nigeria (Akinrele, 1970), *koko* or *akasain* Ghana (Andah and Muller, 1973), *mahewu* in South Africa (Schweigart and de Wit, 1960) and *Poto-poto* in Congo Brazzaville. It contains very poor nutritional value. 47% of the population in rural areas and 78% in Brazzaville feed on *poto-poto (PP)* (Treche *et al.*, 1992). Louémbé *et al.* (2004) had identified the major stages of fermentation and the microbiological diversity

during the fermentation process. Nutritional quality was also documented (Elenga *et al.*, 2009).

Dominant micro organisms are associated with *PP* consists of probiotics bacteria and LAB. Most found are *Lactococcus lactis*, *Lactobacillus plantarum*, *Lb. pentosus*, *Lb. cellobiosus*, *Lb. brevis*, *Leuconostoc mesenteroides*, *Ln. citreum*, *Pediococcus acidilactici*, *Enterococcus* sp., molds and Yeasts (Table 4), were also identified (Louémbé *et al.*, 1996, 2003a; Michel *et al.*, 2012; Abriouel *et al.*, 2006).

Biochemical and micribiological features of bikedi

(bk): In Central Africa, especially in Congo, cassava tuber is consumed mainly as *foufou* (flour), *Chikwangue*, *Bikedi* (Bk), *Mibodo*, *Mayaka ma nkatu* (retted tuber dried and then fermented again), *Gari*, *Mbalampida* (fermented cassava bread with peanut paste), *Mbouata* (tuber retted slurry) and *Minsele* or *Tsaba* (lamellae of cassava tubers cooked and fermented).

In tubers and leaves, *Manihot esculenta* Crantz contains cyanide such cyanogenic glucosides compound like linamarin and lotaustralin (Montgomery, 1980; Dunstan *et al.*, 1906; Kobawila *et al.*, 2005; Louémbé *et al.*, 1997) in a ratio of 93/7 (Butler *et al.*, 1965). Hydrolysis of cyanogenic

Table 4: Isolated microorganisms of *Poto-poto*, food produced by natural fermentation of the maize dough

Raw material	Product	Bacteria	References
Maize (<i>Zea mays</i>)	<i>Poto-poto</i>	Bacteria <i>Acinetobacter calcoaceticus</i> <i>Agrobacterium radiobacter</i> <i>Erwinia</i> sp. <i>Micrococcus varians</i> <i>Staphylococcus sciuri</i> <i>Staphylococcus xylosus</i> <i>Bacillus</i> sp. <i>B. subtilis</i> <i>B. cereus</i> <i>B. macerans</i> <i>Lactobacillus</i> sp. <i>Lb. plantarum</i> <i>Lb. paraplantarum</i> <i>Lb. fermentum</i> <i>Lb. gasserii</i> <i>Lb. delbrueckii</i> <i>Lb. reuteri</i> <i>Lb. casei</i> <i>Lb. brevis</i> <i>Enterococcus</i> sp <i>Lc. lactis</i> <i>Pediococcus acidilactici</i> <i>Leuconostoc</i> sp. <i>Ln. citreum</i> <i>Ln. mesenteroides</i> <i>Enterococcus</i> sp Yeast Molds	Louembé <i>et al.</i> (1996, 2003a), Louembé <i>et al.</i> (2004) and Abriouel <i>et al.</i> (2006)

Table 5: Isolated microorganisms of *Bikédi* and fermented rough of cassava tubers

Raw material	Food	Microorganism	References
Cassaba tubers (<i>Manihotesculenta</i> Crantz)	<i>Bikédi</i> Retted cassava tubers	Bacteria <i>Bacillus</i> sp. <i>B. amyloliquefasciens</i> <i>B. cereus</i> <i>B. polymyxa</i> <i>Enterobacter</i> spp <i>Lb. coprophilus</i> <i>Lb. delbrueckii</i> <i>Lb. fermentum</i> <i>Ln. mesenteroides</i> <i>Ln. lactis</i> <i>Lc. lactis</i> <i>Klebsiella</i> spp. <i>Clostridium butyricum</i> Yeast Molds	Malonga <i>et al.</i> (1996, 1993)

glycosides by endogenous linamarase enzyme releases the toxic hydrogen cyanide.

To remove hydrogen cyanide people practice traditional fermentation including retting and browning (Dunican, 1990; Lancaster *et al.*, 1982; Ongusua *et al.*, 1983). In addition, this allows a significant production of organic acids and a softening of tubers essential for the subsequent operations of transformation (Malonga *et al.*, 1996; Kobawila *et al.*, 2005). In order to improve the quality of final product, studies have been determined the effectiveness of these traditional techniques in reducing of cyanide compounds (Louembé *et al.*, 1997; Kobawila *et al.*, 2005). Many studies have been devoted to microbiology in the study of retting and softening phenomenon (Okafor *et al.*, 1984; Oyewole and Odunfa, 1990; Cooke, 1978; Malonga *et al.*, 1996; Conn, 1969; Ampe *et al.*, 1995),

but the understanding on detoxification is still poor and controversial.

During retting of cassava tubers LAB such *Lc. lactis* is predominant among microorganisms. This species is supplanted between 48 and 96 hours of fermentation by *Ln. mesenteroides* and *Ln. lactis*. At the end of retting, between the third and fourth days, the number is close to 109 bacteria per gram of pulp in cassava (Malonga *et al.*, 1996). LAB are essentially composed of Lactobacilli (73%), *Leuconostocs* (20%) and lactococci (6.7%). The isolated bacteria are *Lactobacillus plantarum*, *Lb. delbrueckii*, *Lb. fermentum*, *Lb. coprophilus*, *Lc. lactis*, *Ln. mesenteroides* and *Ln. lactis* (Table 5) (Louembé *et al.*, 2003b). Many of them have an amylase activity such as *Lb. plantarum* and *Lactococcus* sp., *Bacillus* species including *B. cereus*, *B. amyloliquefasciens*, *B. polymyxa*, Enterobacteriaceae

such as *Klebsiella* sp. and *Enterobacter* sp. (Louembé *et al.*, 2003b) were identified. The linamarasic activity has been observed with *Lc. lactis*, *Ln. mesenteroides*, *Lactobacillus* sp. and *Lb. Plantarum* (Louémbé *et al.*, 1997). *Clostridium butyricum* MGPP179, a specie close to *Clostridium felcium*, was found in retting tubers of cassava (Kéléké *et al.*, 2007).

Biotechnology of Ntoba Mbodi (NM): *NS, PP* and *BK* products are processed in acidic medium, while *Ntoba Mbodi* (NM) is accompanied by a significant alkalization (pH 8.6) of fermentation medium. The fermented NM. Moreover, cassava leaves are used for human consumption due to their high nutritional value. Cassava leaves are highly rich in protein, minerals and vitamins and all essential amino acids except for methionine and phenylalanine (Eggum, 1970; Ravindran and Ravindran, 1988; Gómez and Valdivieso, 1985; Rogers and Milner, 1963; Ross and Enriquez, 1969). The transformation of cassava leaves in NM lasts only four days (Kobawila *et al.*, 2005). Beyond 4 days, NM fermentation appears significantly altered. This had not been observed in acidifying fermentation (Malonga *et al.*, 1993). The alkalization of the pH determines the appearance of the microbial flora. The activity of pectate-lyases, polygalacturonase, pectinesterase, cellulases increases the softening from the first to the third day of NM fermentation (Mokemiabeka *et al.*, 2011). As mentioned above with

Clotridium butyricum, the action of pectin enzymes using polygalacturonate polymethyl substrate released by the microorganism is necessary for softening (Kéléké *et al.*, 2007). The increase of browning is correlating with increase of the concentration of polyphenolic polymers due to the polyphenol oxidases activity. This phenomenon of browning is interesting for NM fermentation since it improves its sensory properties (Martinez and Whitaker, 1995; Whitaker, 1995). Enzymes like proteases, deaminases and decarboxylases which generate amines are responsible for the alkalization in medium (pH 8.5). *B. Subtilis* and *B. amyloliquefasciens* isolated from NM would beinvolved for the pH increasing by producing ammonia released during the degradation of proteins and peptides (Steinkraus, 1991; Ouoba *et al.*, 2015) (Table 5). The alkaline pH promote the development of other *Bacillus* species including *B. pumilus*, to use hydrogen cyanide for their nutrition and therefore to decrease detoxification (Knowles, 1976; Wong and Jackson, 1983). These microorganisms are commonly found in vegetable products (Etchells *et al.*, 1975) and also some species like *Micrococcus varians*, *B. macerans*, *B. subtilis*, *Staphylococcus sciuri* et *Staphylococcus xylosus* (Pederson and Albury, 1983) (Table 5) are the more representatives.

However *Ln. mesenteroides* is mainly met in the initial stage of the NM fermentation (Mukherjee *et al.*, 1983; Garvie, 1986). It is worthy notedthat yeasts are

Table 6: Ntoba Mbodi (NM) isolated microorganisms from the natural fermentation of cassava leaves

Raw material	Foods	Microorganism	References
Cassava leaves	Ntobambodi (Fermented cassava leaves)	<i>Acinetobacter calcoaceticus</i> <i>Agrobacterium radiobacter</i> <i>Bacillus</i> sp. <i>B. amyloliquefasciens</i> <i>B. macerans</i> <i>B. circulans</i> <i>B. subtilis</i> <i>B. pumilus</i> <i>B. polymyxa</i> <i>B. megaterium</i> <i>B. sphaericus</i> <i>B. brevis</i> <i>Erwiniaspp.</i> <i>Lysinibacillus louembei</i> sp. <i>Lactobacillus</i> spp. <i>Lb. fermentum</i> <i>Lb. plantarum</i> <i>Lb. lactisdiacetylactis</i> <i>Micrococcus varians</i> <i>Pediococ cuscerevisiae</i> <i>Pediococ cusacidilactici</i> <i>Staphylococcus sciuri</i> <i>Staphylococcus xylosus</i> <i>Weissella confusa</i> <i>Weissella cibaria</i> <i>Pediococcus pentosaceus</i> <i>Enterococcus</i> sp. <i>E. casseliflavus</i> <i>E. faecium</i> <i>E. faecalis</i> <i>E. avium</i> <i>E. hirae</i>	Louembé <i>et al.</i> (2003b) and Ouoba <i>et al.</i> (2010, 2015)

absent in the fermentation of residual sugars may be due to the high pH on NM fermentation.

Besides microorganisms previously mentioned, *Acinetobacter calcoaceticus*, *Agrobacterium radiobacter*, *Erwinia* spp. *Bacillus cereus*, *Lb. plantarum*, *Lb. fermentum*, *Lactobacillus* spp, *Lactococcus lactis diacetylactis* and *Pediococcus acidilactici* (Table 6) are also found. Recently, a novel spore-forming bacterium was isolated from NM, *Lysinibacillus louembei* sp. nov. (Ouoba *et al.*, 2015). Some of strains isolated from NM have both a pectinolytic and proteolytic activity and others are only one (Kéléké *et al.*, 2007; Kobawila *et al.*, 2005; Nguimbi *et al.*, 2014). Bacteria which may play a significant role in this transformation would be *B. macerans*, *B. subtilis*, *B. amyloliquefasciens*, *B. cereus*, *Staphylococcus xylosus* and *Erwinia* sp. (Peter, 1986; Nguimbi *et al.*, 2014). By degrading pectins and polysaccharides, enzymes promote softening of cassava leaves (Schleifer, 1986; Sheiman *et al.*, 1976) and thereby improving the digestibility of the organic plant.

Amplification of 16S RNA gene and Sequencing experiments of in NM isolates allowed identification some bacteria including *Weissella confusa*, *W. cibaria*, *Lb. plantarum*, *Pediococcus pentosaceus*, *Enterococcus casseliflavus*, *E. faecium*, *E. faecalis*, *E. avium* and *E. hirae* (Ouoba *et al.*, 2010). The presence of this type of bacteria demonstrates the attention that should be brought to NM, regarding different stages of quality control and HACCP procedures. These bacteria are typically found at the beginning of fermentation and some of them are responsible for nosocomial infections (Khan *et al.*, 2015).

The enzymes of biotechnological interest: fibrinolytic and pectinolytic enzymes:

Bacillus sp. has been considered for their capacity to secrete more therapeutic enzymes. Fibrinolytic and pectinolytic enzymes have been identified (Nguimbi and Wu, 2002; Nguimbi *et al.*, 2014; Ouoba *et al.*, 2015). Previous studies have been shown that several bacilli species are able to produce fibrinolytic enzymes including *Bacillus natto*, *B. amyloliquefasciens* DC-4, *Bacillus* sp. CK, *B. subtilis* EM 29 and *B. amyloliquefasciens* CH51 (Sumi *et al.*, 1987; Peng and Zhang, 2002; Kim *et al.*, 1996; Nguimbi and Wu, 2002; Kim *et al.*, 2009). *B. subtilis* and *B. amyloliquefasciens* isolated from NM are able to degrade fibrin and casein (Nguimbi *et al.*, 2014). Deep studies will be specially addressed to *Lysinibacillus louembei*, novel exciting bacteria (Ouoba *et al.*, 2015). Softening of cassava leaves is the main stage which indicates the end of the fermentation. *Clostridium bytirycum* MGPP179 genetically close to *Clostridium felcium* seems to have the necessary assets for his involvement in the retting process of cassava tubers and softening (Kéléké *et al.*, 2007). Several bacteria secrete enzymes degrading cell walls (Nambisan and

Sundaresan, 1985). Alteration of pectic compounds is generally related to the action of pectinolytic enzymes that generate significant changes in cell structures. This is especially saponifying enzymes like pectin lyase, pectate lyase, polygalacturonase and some unknown hydrolases that can hydrolyze pectin containing in cell wall (Rexová-Benková and Markovic, 1976; Thibault, 1980). These enzymes allow the destruction of plant tissues and therefore the opening of target compartments thus favoring contact between the endogenous linamarase and linamarin (Okafor *et al.*, 1984; Nambisan and Sundaresan, 1985). Microorganisms associated in pectinolytic activities and the evolution of softening were therefore studied. Studies showed that many bacteria are able to synthesize linamarase enzyme in the presence of appropriate linamarin or lotaustralin substrate. This includes *Lc. lactis*, *Ln. mesenteroides* and *Lb. plantarum* (Okafor and Ejiofor, 1985).

Bacteriocins and probiotic bacteria of congo brazzaville fermented foods:

Bacteriocins are antimicrobial peptides produced by bacteria and could present a bactericidal effect against closely related species (Cotter *et al.*, 2005). LAB represents a model of choice to ensure the absence pathogenic bacteria in the final product (Konings *et al.*, 2000). Nisin from LAB is the only bacteriocin approved for use as a food preservative (Balciunas *et al.*, 2013). Thirty-one bacteriocins from *Lactobacillus* specie were identified in Congolese fermented maize product (*Poto-poto*). *Lactobacillus* sp. can inhibit pathogenic bacteria including *Escherichia coli*, *Salmonella enterica*, *Enterobacter aerogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Enterococcus faecalis*. Specific studies were performed on the correlation and the involvement between LAB fermentation and the reduction of pathogenic bacteria such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli* and *Shigella* sp. Children fed with fermented starches showed very positive effects by reducing the risk of childhood diarrhea (Svangberg, 1991a, 1991b; Svangberg and Svanberg, 1989).

Fermented foods like NM, PP and BK are a best ecological niche to isolate microorganism producing bacteriocins. The new strain *Lysinibacillus louembei* isolated from NN could be interesting to investigate (Ouoba *et al.*, 2015).

Promising and sustainable future: The use of bacteriocin-producing bacteria may be interesting as well as economic and legislative level. Producing bacteria bacteriocins may be added as a starter in fermented products (Gálvez *et al.*, 2007; Dortu and Thonart, 2009). Their use must consider the *Quorum Sensing* System, the product composition (available nutrients, pH and food additives) and storage

conditions. Bacteria with pectinolytic and linamarase activities isolated from Congo Brazzaville traditional fermented foods are particularly interesting as a starter in fermented products. The characterization of these enzymes, from gene isolation to the final product will be an essential biotechnological tool for use in Biocatalysis. New isolated strains such as *Lysinibacillus louembei* could be interesting for the discovery of new molecules of biotechnological interest.

CONCLUSION

Basing on numerous studies discussing and mentioned above, we conclude that Congo Brazzaville fermented foods contain a fascinating diversity of microorganism. Many of them have a biotechnological interest and therapeutic properties. In addition molecules delivered from bacteria could be a great use to humanity and the natural world for a sustainable future. This discovery opens a promising future for the valuation through research and development.

RÉFÉRENCES

- Abriouel, H., N. Ben Omar, R.L. López, M. Martínez-Cañamero, S. Keleke and A. Gálvez, 2006. Culture-independent analysis of the microbial composition of the African traditional fermented foods *poto poto* and *dégué* by using three different DNA extraction methods. *Int. J. Food Microbiol.*, 111(3): 228-233.
- Akingbala, J.O., L.W. Rooney and J.M. Faubion, 1981. A laboratory procedure for the preparation of *ogi*, a Nigerian fermented food. *J. Food Sci.*, 46(5): 1523-1526.
- Akinrele, I.A., 1970. Fermentation studies on maize during the preparation of a traditional African starch-cake food. *J. Sci. Food Agr.*, 21(12): 619-625.
- Ampe, F., S. Keleke, H. Robert and B. Alain, 1995. The Role and Origin of Pectin Degrading Enzymes during Cassava Retting. In: Agbor Egbe, T., A. Brauman, D. Griffon and S. Trèche (Eds.), *Transformation Alimentaire du Manioc*. ORSTOM, Paris, pp: 331-344.
- Andah, A. and H.G. Muller, 1973. Studies on *koko*, a Ghanaian fermented maize porridge. *Ghana J. Agric. Sci.*, 6: 103-108.
- Balciunas, E.M., F.A.C. Martinez, S.D. Todorov, B.D.G. de Melo Franco, A. Converti and R.P. de Souza Oliveira, 2013. Novel biotechnological applications of bacteriocins: A review. *Food Control*, 32(1): 134-142.
- Bassir, O., 1962. Observations on the fermentation of palm-wine. *West Afr. J. Biol. Chem.*, 6: 5-21.
- Butler, G.W., R.W. Bailey and L.D. Kennedy, 1965. Studies on the glucosidase "linamarase". *Phytochemistry*, 4(3): 369-381.
- Conn, E.E., 1969. Cyanogenic glycosides. *J. Agr. Food Chem.*, 17(3): 519-526.
- Cooke, R.D., 1978. An enzymatic assay for the total cyanide content of cassava (*Manihot esculenta* Crantz). *J. Sci. Food Agr.*, 29(4): 345-352.
- Cotter, P.D., C. Hill and R.P. Ross, 2005. Bacterial lantibiotics: Strategies to improve therapeutic potential. *Curr. Protein Pept. Sc.*, 6(1): 61-75.
- Dortu, C. and P. Thonart, 2009. Les bactériocines des bactéries lactiques: Caractéristiques et intérêts pour la bioconservation des produits alimentaires. *Biotechnol. Agron. Soc. Environ.*, 13(1): 143-154.
- Dunican, L.K., 1990. Strategies for developing the cassava industry. Nuclear and related techniques in the improvement of traditional fermentation processing of cassava. International Atomic Energy Agency, Vienna, Austria, pp: 9-14.
- Dunstan, W.R., T.A. Henry and S.J.M. Auld, 1906. Cyanogenesis in plants. Part V.--The occurrence of phaseolunatin in cassava (*Manihot aipi* and *Manihot utilissima*). *P. R. Soc. Lond. B-Conta.*, 78(523): 152-158.
- Eggum, B.O., 1970. The protein quality of cassava leaves. *Brit. J. Nutr.*, 24: 761-768.
- Elenga, M., J. Massamba, S. Kobawila, V. Makosso and T. Silou, 2009. Evaluation et amélioration de la qualité nutritionnelle des pâtes et des bouillies de maïs fermenté au Congo. *Int. J. Biol. Chem. Sci.*, 3(6): 1274-1285.
- Etchells, J.L., H.P. Fleming and T.A. Bell, 1975. Factors Influencing the Growth of Lactic Acid Bacteria During Brine Fermentation of Cucumbers. In: Carr, J.G., C.V. Cutting and G.C. Whiting (Eds.), *Lactic Acids Bacteria in Beverages and Food*. Academic Press, New York, pp: 281-305.
- Faparusi, S.I., 1973. Origin of initial microflora of palm wine from oil palm trees (*Elaeis guineensis*). *J. Appl. Microbiol.*, 36(4): 559-565.
- Faparusi, S.I. and O. Bassir, 1972. Factors affecting the quality of palm-wine 2. Period of storage. *W. Afr. J. Biol. Appl. Chem.*, 15: 24-28.
- Gálvez, A., H. Abriouel, R.L. López and N. Ben Omar, 2007. Bacteriocin-based strategies for food biopreservation. *Int. J. Food Microbiol.*, 120(1-2): 51-70.
- Garvie, E.I., 1986. Genus *Leuconostoc*. Vol. 2, In: Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.), *Bergey's Manual of Systematic Bacteriology*. Williams and Wilkins, Baltimore, pp: 1071-1075.
- Gómez, G. and M. Valdivieso, 1985. Cassava foliage: Chemical composition, cyanide content and effect of drying on cyanide elimination. *J. Sci. Food Agr.*, 36(6): 433-441.
- Kéléké, S., M.J. Butel and A. Rimbault, 2007. Study of the phenomenon of cassava roots softening during fermentation. *Actes de l'Atelier "Potentialités à la transformation du manioc en Afrique de l'Ouest"*-Abidjan, pp: 275-284.

- Khan, H.A., A. Ahmad and R. Mehboob, 2015. Nosocomial infections and their control strategies. *Asian Pac. J. Trop. Biomed.*, 5(7): 509-514.
- Kim, G.M., A.R. Lee, K.W. Lee, J.Y. Park, J. Chun, J. Cha, Y.S. Song and J.H. Kim, 2009. Characterization of a 27 kDa fibrinolytic enzyme from *Bacillus amyloliquefaciens* CH51 Isolated from Cheonggukjang. *J. Microbiol. Biotechnol.*, 19(9): 997-1004.
- Kim, W., K. Choi, Y. Kim, H. Park, J. Choi, Y. Lee, H. Oh, I. Kwon and S. Lee, 1996. Purification and characterization of a fibrinolytic enzyme produced from *Bacillus* sp. strain CK 11-4 screened from Chungkook-Jang. *Appl. Environ. Microbiol.*, 62(7): 2482-2488.
- Knowles, C.J., 1976. Microorganisms and cyanide. *Bacteriol. Rev.*, 40(3): 652-680.
- Kobawila, S.C., D. Louembe, S. Keleke, D.J. Hounhouigan and C. Gamba, 2005. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. *Afr. J. Biotechnol.*, 4(7): 689-696.
- Konings, W.N., J. Kok, O.P. Kuipers and B. Poolman, 2000. Lactic acid bacteria: The bugs of the new millennium. *Curr. Opin. Microbiol.*, 3(3): 276-282.
- Lancaster, P.A., J.S. Ingram, M.Y. Lim and D.G. Coursey, 1982. Traditional cassava-based foods: Survey of processing techniques. *Econ. Bot.*, 36(1): 12-45.
- Louembé, D., S.C. Kobawila, J.P. Bayenda, V. Mania, O. Michaux, M. Larpent and M. Malonga, 1986. Etude préliminaire de la microbiologie du vin de palme elaeis guineensis. *Microbiol. Alim. Nutr.*, 4: 75-76.
- Louembé, D., A. Brauman, F. Tchicaya and S.C. Kobawila, 1996. Microbiological and biochemical studies of the maize porridge poto-poto. *Microbiol. Alim. Nutr.*, 14: 245-253.
- Louembé, D., M. Malonga, S.C. Kobawila and O. Mavoungou, 1997. Evolution de la teneur en composés cyanés des tubercules de manioc au cours du ouissage-Activité linamarasique de bactéries lactiques. 25: 53-60.
- Louembé, D., S. Kéléké, S.C. Kobawila and J.P. Nzounzi, 2003a. Bactéries lactiques de la pâte fermentée de maïs au Congo. *Tropicultura*, 21: 3-9.
- Louembé, D., S.C. Kobawila, G. Bouanga Kalou and S. Kéléké, 2003b. Etude microbiologique des feuilles fermentées de manioc: "Ntoba Mbodi". *Tropicultura*, 21(3): 106-111.
- Louembé, D., S. Kéléké, S.C. Kobawila and J.P. Nzoussi, 2004. Variabilité et amélioration de la technologie traditionnelle de production de la pâte fermentée de maïs au Congo. *Tropicultura*, 22(4): 211-218.
- Malonga, M., O. Mavoungou, S.C. Kobawila, D. Louembé and A. Brauman, 1993. Les bactéries lactiques au cours du rouissage: Caractérisation et évolution. *Microbiol. Alim. Nutr.*, 11: 471-475.
- Malonga, M., O. Mavoungou, S.C. Kobawila and D. Louembé, 1995. Palm wine: Microbiological and biochemical study in republic of Congo. *Microbiol. Alim. Nutr.*, 13: 195-200.
- Malonga, M., O. Mavoungou, S. Kéléké, S.C. Kobawila and D. Louembé, 1996. Microbiological and biochemical characteristics of Cassava retting. 14: 73-82.
- Martinez, M.V. and J.R. Whitaker, 1995. The biochemistry and control of enzymatic browning. *Trends Food Sci. Tech.*, 6(6): 195-200.
- Miambi, E., J.P. Guyot and F. Ampe, 2003. Identification, isolation and quantification of representative bacteria from fermented cassava dough using an integrated approach of culture-dependent and culture-independent methods. *Int. J. Food Microbiol.*, 82(2): 111-120.
- Michel, E., M. Joachim and S. Thomas, 2012. Effet de l'incorporation de malt sur la fluidité et la densité énergétique des bouillies de maïs-arachide destinées aux nourrissons et aux jeunes enfants. *J. Appl. Biosci.*, 55: 3995-4005.
- Mokemiabeka, S., J. Dhellot, S.C. Kobawila, P. Diakabana, R.N. Ntietie Loukombo, A.G. Nyanga-Koumou and D. Louembe, 2011. Softening and mineral content of Cassava (*Manihot esculenta* Crantz) leaves during the fermentation to produce *Ntoba mbodi*. *Adv. J. Food Sci. Technol.*, 3(6): 418-423.
- Montgomery, R.D., 1980. Cyanogens. In: Liener, I.E. (Ed.), *Toxic Constituents of Plant Foodstuffs*. Academic Press, New York, pp: 143-160.
- Mukherjee, S.K., D.R. Chaudhuri and H. Gangopadhyay, 1983. Studies on Sauerkraut as a Fermented Food of India. In: Steinkraus, K.H. (Ed.), *Handbook of Indigenous Fermented Foods*. Marcel Dekker, New York, pp: 109-114.
- Nambisan, B. and S. Sundaresan, 1985. Effect of processing on the cyanoglucoside content of cassava. *J. Sci. Food Agr.*, 36(11): 1197-1203.
- Nguimbi, E. and Z.R. Wu, 2002. Production of a new fibrinolytic enzyme, bacterium growth and enzyme production conditions, purification and characterization of the new enzyme. *Biotechnology*, 12(2).
- Nguimbi, E., G. Ahombo, R. Moyon, R. Ampa, A. Vouidibio, E.N. Otsira, S.C. Kobawila and D. Louembe, 2014. Optimization of Growth, Fibrinolytic Enzyme Production and PCR Amplification of Encoding Fibrinolytic Enzyme Gene in *Bacillus amyloliquefaciens* Isolated from Ntoba mbodi at Brazzaville. *Int. J. Sci. Res.*, 3(11): 2799-2803.

- Okafor, N., 1972a. Palm-wine yeasts from parts of Nigeria. *J. Sci. Food Agr.*, 23(12): 1399-1407.
- Okafor, N., 1972b. The sources of the microorganism in palm wine. *Nigerian Soc. Microbiol. Proc.*, 1: 102-106.
- Okafor, N., 1975a. Microbiology of Nigerian palm wine with particular reference to bacteria. *J. Appl. Microbiol.*, 38(2): 81-88.
- Okafor, N., 1975b. Preliminary microbiological studies on the preservation of palm wine. *J. Appl. Microbiol.*, 38(1): 1-7.
- Okafor, N. and M.A.N. Ejiofor, 1985. The linamarase of *Leuconostoc mesenteroides*: Production, isolation and some properties. *J. Sci. Food Agr.*, 36(8): 669-678.
- Okafor, N., B. Ijioma and C. Oyolu, 1984. Studies on the microbiology of cassava retting for foo-foo production. *J. Appl. Microbiol.*, 56(1): 1-13.
- Okolie, P.I., C.N. Opara, E.C. Emerenini and S.V.A. Uzochukwu, 2013. Evaluation of bacterial diversity in palm wine by 16S rDNA analysis of community DNA. *Nigerian Food J.*, 31(1): 83-90.
- Olanrewaju, O.O., O.O. Victor and T.A. Titilayo, 2009. Safety of small-scale food fermentations in developing countries. *Internet J. Food Safety*, 11: 29-34.
- Ongusua, O., N. Okafor, O.O. Onyekwere and I.A. Akinrele, 1983. Nigerian Garri. In: Steinkraus, K.H. (Ed.), *Handbook of Indigenous Fermented Foods*. Marcel Dekker, Basingstoke, New York, 9: 208-220.
- Ouoba, L.I., C.A. Nyanga-Koumou, C. Parkouda, H. Sawadogo, S.C. Kobawila, S. Keleke, B. Diawara, D. Louembe and J.P. Sutherland, 2010. Genotypic diversity of lactic acid bacteria isolated from African traditional alkaline-fermented foods. *J. Appl. Microbiol.*, 108(6): 2019-2029.
- Ouoba, L.I., A.B. Vouidibio Mbozo, L. Thorsen, A. Anyogu, D.S. Nielsen, S.C. Kobawila and J.P. Sutherland, 2015. *Lysinibacillus louembei* sp. nov., a spore-forming bacterium isolated from Ntoba mbodi, alkaline fermented leaves of cassava from the Republic of the Congo. *Int. J. Syst. Evol. Microbiol.*, 65(11): 4256-4262.
- Oyewole, O.B. and S.A. Odunfa, 1990. Characterization and distribution of lactic acid bacteria in *Cassava fermentation* during fufu production. *J. Appl. Microbiol.*, 68(2): 145-152.
- Pederson, C.S. and M.N. Albury, 1983. Control of Fermentation. In: Steinkraus, K.H. (Ed.), *Handbook of Indigenous Fermented Foods*. Marcel Dekker, New York, pp: 102-108.
- Peng, Y. and Y.Z. Zhang, 2002. Isolation and characterization of fibrinolytic enzyme producing strain DC-4 from Chinese douche and primary analysis of the enzyme property. *Chinese High Technol. Lett.*, 12: 30-34.
- Peter, H.A.S., 1986. Endospore-Forming Gram-Positive Rods and Cocci. In: Peter, H.A.S., S.M. Nicholas and M. Sharpe Elisabeth (Eds.), *Bergey's Manual of Systematic Bacteriology*. Williams et Wilkins, Baltimore, 2: 1104-1207.
- Ravindran, G. and V. Ravindran, 1988. Changes in the nutritional composition of cassava (*Manihot esculenta Crantz*) leaves during maturity. *Food Chem.*, 27(4): 299-309.
- Rexová-Benková, L. and O. Markovic, 1976. Pectic enzymes. *Adv. Carbohyd. Chem. Biochem.*, 33: 323-385.
- Rogers, D.J. and M. Milner, 1963. Amino acid profile of manioc leaf protein in relation to nutritive value. *Econ. Bot.*, 17(3): 211-216.
- Ross, E. and F.Q. Enriquez, 1969. The nutritive value of cassava leaf meal. *Poultry Sci.*, 48(3): 846-853.
- Schleifer, K.H., 1986. Gram-positive Cocci. In: Peter, H.A.S., S.M. Nicholas and M. Sharpe Elisabeth (Eds.), *Bergey's Manual of Systematic Bacteriology*. Williams et Wilkins, Baltimore, 2: 999-1103.
- Schweigart, F. and J.P. de Wit, 1960. Favourite beverage of the bantu. Preparing and drying of mahewu and its nutritional value. *Food Ind. South Afr.*, 12: 25-27.
- Sheiman, M.I., J.D. Macmillan, L. Miller and T. Chase Jr., 1976. Coordinated action of pectinesterase and polygalacturonate Lyase complex of *Clostridium multifementans*. *Eur. J. Biochem.*, 64(2): 565-572.
- Steinkraus, K.H., 1991. African Alkaline Fermented Foods and Their Relation to Similar Foods in Other Parts of the World. In: Wesby, R., P.J.A. Reilly (Eds.), *Traditional African Foods: Quality and Nutrition*. Marcel Dekker, New York, pp: 87-92.
- Sumi, H., H. Hamada, H. Tsushima, H. Mihara and H. Muraki, 1987. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese natto; a typical and popular soybean food in the Japanese diet. *Experientia*, 43(10): 1110-1111.
- Svangberg, U., 1991a. Lactic Fermentation of Cereal-Based Weaning Gruels and Improved Nutritional Quality. In: Westby, A. and P.J.A. Reilly (Eds.), *Traditional African Foods: Quality and Nutrition*. International Foundation of Science (IFS), Sweden, pp: 53-60.
- Svangberg, U., 1991b. The Potential Role of Fermented Cereal Gruels in Reduction of Darrhorea among Young Children. In: Westby, A. and P.J.A. Reilly (Eds.), *Traditional African Foods: Quality and Nutrition*. International Foundation of Science (IFS), Sweden, pp: 33-38.
- Svangberg, U. and A.S. Svanberg, 1989. Improved Iron Availability in Weaning Foods using Germination and Fermentation. In: Southgate, D.A.T., I.T. Johnson and G.R. Fenwick (Eds.), *Nutrien Availability: Chemical and Biological Aspect*. Royal Society of Chemical, Spetial Publication No. 72, pp: 179-181.

- Thibault, J.F., 1980. Biochimie Appliquée. Gauthier-Villars, Paris, pp: 323-385.
- Treche, S., P. Giamarchi, S. Pezennec, G. Gallon and J. Massamba, 1992. Les bouillies de sevrage au Congo: Composition, valeur nutritionnelle et modalités d'utilisation. 5ème Journées Internationales du GERM, Montpellier, Novembre 22-27, pp: 1-10.
- Van Pee, W. and J.G. Swings, 1971. Chemical and microbiological studies on Congolese palm wines. (*Elaeis guineensis*). E. Afr. Agric. Food J., 36(3): 311-314.
- Whitaker, J.R., 1995. Polyphenol Oxidase. In: Wong, D.W.S. (Ed.), Food Enzyme: Structure and Mechanism. Springer US, New York, pp: 271-307.
- Wong, P.P.W. and H. Jackson, 1983. Chinese Hum Choy. In: Steinkraus, K.H. (Ed.), Handbook of Indigenous Fermented Food. Marcel Dekker, New York, pp: 118-119.