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Physiological Characterization of *Staphylococci* and *Micrococci* Isolated from Fermented Cassava Leave (*Manihot esculenta Crantz*), *Ntoba mbodi*

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A B S T R A C T

The Ntomba mbodi is a food who is consumed in the Republic of Congo. It is obtained by fermentation of cassava leaves. During the fermentation of cassava leaves develop several genera or species of fermentative bacteria. However other non-fermentative bacteria may be present. In order to study microbial diversity in the fermentation of cassava leaves, *Staphylococci* and *Micrococci* were isolated and their physiological and biochemical characteristics were followed during growth. . The identification of *Staphylococci* and *Micrococci* was made from the cultural characters. The various staphylococci were identified were using API Staph gallery. The tube culture method was used to determine the respiratory type. Bacterial growth was followed as a function of pH and NaCl concentration. These parameters were analyzed as a function of fermentation time. The identification results are given: 100% *Micrococcus* spp. 50%, *Staphylococcus sciuri*, 40% of *Staphylococcus xylosus* and respectively 5% of *Staphylococcus chromogen* and *Staphylococcus lentus*. The research of respiratory type gave the following break down: 64.5% of bacteria were strict aerobic and 35.5% aerobic-anaerobic. The determination of the growth parameters showed that strains grow at alkaline pH 8.6 and tolerate effective ' 15% NaCl. However, some strains are tolerant of acidic pH (pH 3.5) and grow at low salt concentrations.

Introduction

Nutrition is the report of a balance between man and the natural resources available. In Congo, to meet the food needs and improve the nutritional quality of food, more fruits and vegetables are processed .Among these vegetable ago cassava leaves. Cassava leaves (Manihot esculenta Crantz) occupy an important place in the Congolese diet. They are prepared according to several recipes and thereby constitute the most consumed vegetables because of their fiber 16-26% protein and 17-34%, which gives them a high nutritional value. Thus, to enhance and diversify revenues, cassava leaves are subjected to fermentation process, traditionally made in the craft or family

units (Louembe, Kobawila *et al.*, 2003).

This fermentation allows the production of a final product of better nutritional quality and organoleptic called ntoba mbodi the work of microbial activity (Chauvin 2005) previous studies on the ntoba mbodi showed the presence of several bacterial species *Bacillus subtilis, Bacillus. macerans, Bacillus cereus, Micrococcus varians, Staphylococcus sciuri* and *Straphylococcus xylosus*. These microorganisms are partly responsible for the production of enzymes for the transformation of cassava leaves (Kobawila C. S. 2004; Leclere *et al.*, 2013-2014). It is in this light that we were interested in the physiological characterization and Staphylococci Micrococci fermented cassava leaves.

Materials and Methods

The plant material was essentially manioc leaves. Ntoba mbodi samples used came from the workshops of craft production, markets of Brazzaville and Pointe-Noire as well as those prepared in the laboratory according to the craft process. The extracts were prepared from 10 grams of fermenting leaves. The leaves were crushed and homogenized in Maximum Recovery diluent CMO733. The mother solution is subjected to a range of decimal dilution seeding. The extracts were inoculated by spreading on Baird Parker media (CM0275) at pH 6.8 and Chapman (CM0085) at pH 7.5 and then incubated at 30°C for 24 to 48 hours. Physiological tests were performed with nutrient agar (CM0003) at pH 7.5 and Nutrient broth at pH 7.5.

The genera *Staphylococcus* and *Micrococcus* were identified according to the protocol of the 4th Edition Bacteriological Techniques Manual (1974). The type of respiration was determined by three cultivation methods tube: on the surface, at the bottom and along the tube. The identification was made by a gallery Api Staph (Brun *et al.*, 2002).

Different strains have been grown in the tubes containing nutrient broth with increasing concentrations of NaCl 4%, 6%, 8%, 10%, 12%, 15% and at different pH of 3.5, 5, 7, 8 and 9. the tubes were respectively incubated in an incubator for 24 to 96 hours and 24 to 72 hours. The growths in the tubes were observed every 24 hours in comparison with the turbidity of the control tubes. The presence of staphylococci and micrococci is confirmed in the tubes respectively containing the nutrient broth at pH given unseeded, nutrient broth with NaCl without culture and nutrient broth with culture without NaCl.

Results and Discussion

During this study, 31 strains were isolated during the fermentation. The identification gave 11 *micrococci* strains (35.5%) and 20 staphylococcal strains (64.5%). *Staphylococcus* spp and *Micrococus* identified and their frequencies are shown in Figure.1. The staphylococci included *Staphylococcus sciuri* (50%), *S. xylosus* (40%), *S. lentus* chromogen and respectively (5%) while for micrococci there were only *Micrococcus* spp (100%). The physiological analysis gave 64.5% of aerobic-anaerobic-optional strains and 35.5% of strict aerobic (Tables I and II).

The study of growth based on the medium pH showed that 100% of strains were growing in alkaline medium; 36% of *Micrococcus* ssp strains and 5% *S. sciuri* grew at pH 5. However, no growth was observed at pH 3.5 (Tables III and IV). These results confirm those in the literature showing that the majority of non-lactic fermentation bacteria tolerate alkaline pH (Aurélia Hiron 2013, Zulfakar SS *et al.*,2013).

The analysis of growth depending on the concentration of sodium chloride showed that all *Staphylococcus* strains (100%) growing at 4%, 6%, 8%, 10%, 12% and 15% while the strains *Micrococcus* tolerate concentrations of 4%, 6%, 8% and 10%. However, with high concentrations of 12 to 15% NaCl growth respectively (9.1%) and (18.2%) *Micrococcus spp* a strain is inhibited (Table IV).

The result of the determination of the type of breathing helped to highlight the distinctiveness between Staphylococci kinds of aerobic-anaerobic-optional kind and strict aerobic type Micrococci (Bacteriological 4th Edition Technical Manual, 1974. Brun *et al.*, 2002).

The identification techniques show biodiversity and frequency of Staphylococci and Micrococci isolated during the alkaline fermentation. In addition to *Staphylococcus xylosus* and *Staphylococcus sciuri* identified in previous studies (Louembe D., S .C. Kobawila, *et al.*,, 2003), there are the chromogenic species *Staphylocuccus* and *Staphylococcus* l*entus* all belonging to SCN (coagulase-negative staphylococci) (Euzeby J. P.1997) .

Table.1 Respiratory type of *Staphylococci*

Samples	Isolated strains	Number	Respiratory Type
Market Brazzaville	*S. sciuri* *S. Xylosus*	1 1	AAF AAF
Market Pointe-Noire	*S. Lentus,* *S. Xylosus*	1 1	AAF AAF
E1	*S. sciuri,* *S. Xylosus*	1 1	AAF AAF
E2	*S. sciuri,* *S. Xylosus*	1 1	AAF AAF
E3	*S. sciuri* *S. Xylosus*	3 2	AAF AAF
E4	*S. chromogène* *S. sciuri* *S. Xylosus*	1 4 2	AAF AAF AAF

Legend: AAF: aerobic anaerobic optional
E1, E2, E3, E4: Laboratory samples at 0, 24, 48 and 72 hours of fermentation

Table.2 Respiratory type of *Micrococci*

Samples	Isolated strains	Number	Respiratory type
Market Brazzaville	*Micrococcus spp*	1	AS
Market Pointe-Noire	*Micrococcus spp*	1	AS
E1	*Micrococcus spp*	4	AS
E2	*Micrococcus spp*	2	AS
E3	*Micrococcus spp*	2	AS
E4	*Micrococcus spp*	1	AS

Legend: AS: Strict aerobic ; E1, E2, E3, E4: Laboratory samples at 0, 24, 48 and 72 hours of fermentation

Table.3 Staphylococcal growth at different pH

Samples	Isolated strains	Number	pH of growth				
			3,5	5	7	8	9
Market Brazzaville	*S. Xylosus* *S. sciuri*	1 1	_	_	+	+	+
Market Pointe-Noire	*S. Lentus* *S. xylosus*	1 1	_	_	+	+	+
E1	*S. sciuri* *S. Xylosus*	1 1	_ _	+ _	+ +	+ +	+ +
E2	*S. sciuri,* *S. xylosus*	1 1	_	_	+	+	+
E3	*S. sciuri,* *S. xylosus*	3 2	_	_	+	+	+
E4	*S. chromogène,* *S. sciuri,* *S. xylosus*	1 4 2	_	_	+	+	+

Legend: E1, E2, E3 , E4: Laboratory samples at 0, 24, 48 and 72 hours of fermentation; **-:** no growth; **+:** growth

Table.4 Micrococci growth at different pH

Samples	Isolatde strains	Number	growth at different pH				
			3, 5	5	7	8	9
Market Brazzaville	*Micrococcus spp*	1	_	+	+	+	+
Market Pointe-Noire	*Micrococcus spp*	1	_	_	+	+	+
E1	*Micrococcus spp*	2	_	_	+	+	+
	Micrococcus spp	2	_	+	+	+	+
E2	*Micrococcus spp*	2	_	+	+	+	+
E3	*Micrococcus spp*	2	_	_	+	+	+
E4	*Micrococcus spp*	1	_	_	+	+	+

Legend: E1, E2, E3 , E4: Laboratory samples at 0, 24, 48 and 72 hours of fermentation; **-:** no growth; **+:** growth

Table.5 Growth of *Staphylococci* at different concentrations of NaCl (%)

Samples	Isolated strains	Number	Growth at different concentrations of NaCl (%)					
			4%	6%	8%	10%	12%	15%
Market Brazzaville	*S. sciuri* *S. xylosus*	1 1	+	+	+	+	+	+
MarketPointe-Noire	*S. lentus* *S. xylosus*	1 1	+	+	+	+	+	+
E1	*S. sciuri,* *S. xylosus*	1 1	+	+	+	+	+	+
E2	*S. sciuri,* *S. xylosus*	1 1	+	+	+	+	+	+
E3	*S. sciuri* *S. xylosus*	2 2	+	+	+	+	+	+
E4	*S. chromogène* *S. sciuri,* *S. xylosus*	1 4 2	+	+	+	+	+	+

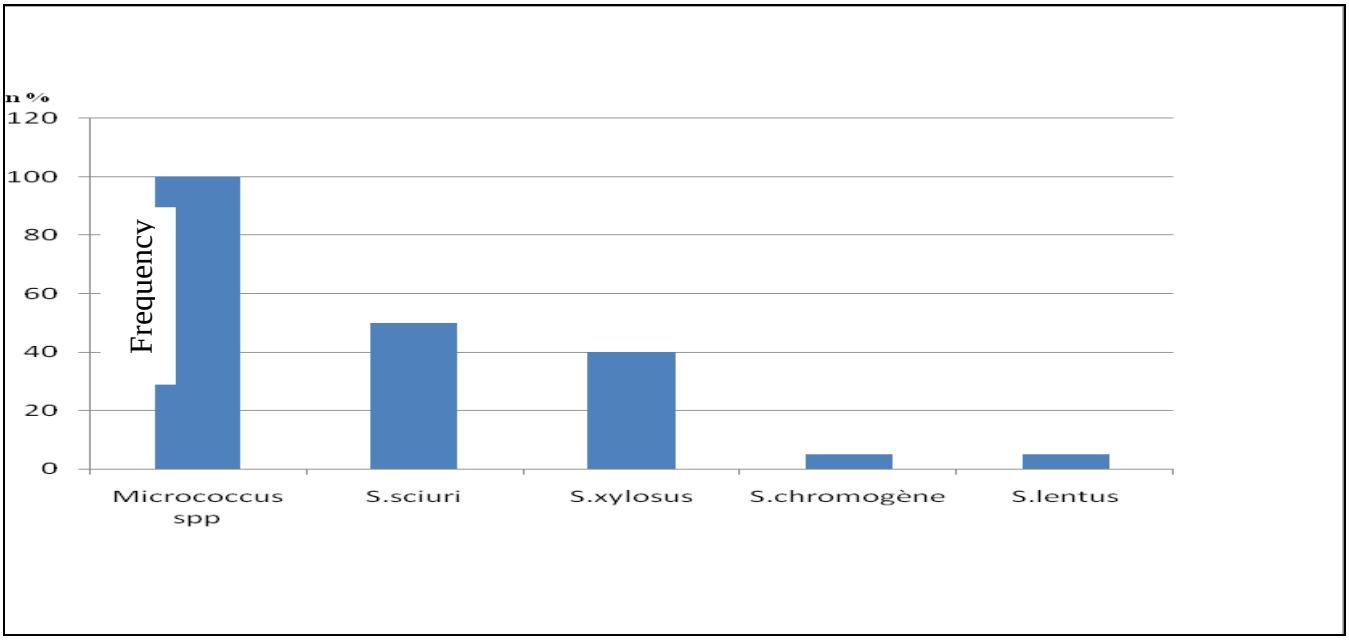
Legend: E1, E2, E3 , E4: Laboratory samples at 0, 24, 48 and 72 hours of fermentation; **-:** no growth; **+:** growth

Table.6 Growth of *Micrococci* at different concentrations of NaCl (%)

Samples	Isolates strains	Number	Growth at different concentrations of NaCl (%)					
			4%	6%	8%	10%	12%	15%
Market Brazzaville	*Micrococcus spp*	1	+	+	+	+	+	+
Market Pointe-Noire	*Micrococcus spp*	1	+	+	+	+	+	+
E1	*Micrococcus spp*	3	+	+	+	+	+	+
	Micrococcus spp	1	+	+	+	+	+	_
E2	*Micrococcus spp*	1	+	+	+	+	+	+
	Micrococcus spp	1	+	+	+	+	_	_
E3	*Micrococcus spp*	2	+	+	+	+	+	+
E4	*Micrococcus spp*	1	+	+	+	+	+	+

Legend: E1, E2, E3 , E4: Laboratory samples at 0, 24, 48 and 72 hours of fermentation; **-:** no growth; **+:** growth

Fig.1 Frequency of strains of *Staphylococcus* and *Micrococcus* in Ntoba mbodi



Bacteria isolated

Traduction machinelle

Several studies have revealed the presence of staphylococcal strains in fermented foods. *Micrococcus spp* and *Staphylococcus xylosus* found in all samples is the most abundant microflora. They have also been isolated in dry fermented sausages and milk when they participate in the biochemical transformation (Nychas *et al.*, 1990, Stephanie Corbiere *et al.*,2006, Branger *et al.*,2007).

Their high frequency suggests that these species participate in the process of fermentation by degrading the basic constituents with production necessary metabolites nutritionally (Frenney *et al*, 2008). Chromogenic species *Staphylococcus* and *Staphylococcus lentus* which are small proportion are unusual species of plants fermented products. They were isolated from dairy products as they help improve the organoleptic quality of food (Hermen *et al.*,, 1992; Stadie *et al.*, 2013).

The physico-chemical point of view, all the isolated strains, except for 18.2% of *Micrococcus spp*, are resistant up to 15% sodium chloride (NaCl). This is one of the characteristics of *Staphylococcus xylosus* isolated from other fermented foods such as dry-sausage (Coppola *et al.*,, 1997; Montel *et al*, .1992).

In conclusion, the use of identification techniques was an excellent alternative in our validation study, showing the biodiversity of staphylococci and micrococci and their frequency in the fermentation of cassava leaves. Physiology strains allowing distinguishing between the two types with the application of growth at different pH tests and varying concentrations of sodium chloride (NaCl) showed that the microbial population constituting the flora is *Staphylococcus* and *Micrococcus* able to adapt to the conditions of production and can be more competitive during fermentation.

Thus, the study has shown the wide variety and diversity of bacterial groups that adopt different strategies to adapt to the various conditions for their survival and, consequently, change their metabolism: pH tolerance to acidic or basic, the ability to grow to high concentrations of NaCl.

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