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Study of Biodiversity and Physiological Characters of Lactic Bacteria Isolated in Fermented Pepper (*Capsicum fructescens*)

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A B S T R A C T

The pepper (*Capsicum fructescens*) is a condiment used in human food. In order to demonstrate the presence of lactic bacteria and their biodiversity in the fermentation of peppers, 33 lactic bacteria were isolated from the fermented pepper samples sold in the markets of Brazzaville and samples fermented in the laboratory. The pH was measured every 24 hours from the fermenting peppers homogenized in distilled water. The inoculated cultured were prepared from 10 grams of fermenting chili for 24 hours, homogenized in maximum recovery diluent CMO733 pH 7.0. The decimal dilutions of 10^{-1} to 10^{-6} were made from the suspension obtained. Isolation, purification and enumeration were made of 0.1ml seeded suspension on MRS CM0361 pH 6, 2. Three Petri dishes were inoculated for each dilution and incubated for 18 to 24 hours in an oven at 37 ° C. The morphological and physiological identification tests were performed on different cultures. The different bacteria isolated growth parameters were determined using the temperature variation ranges of pH and NaCl concentration. The results obtained have shown that 84.85% of the bacteria were of the genus *Lactobacillus*, *Lactococcus* of 12.12% and 3.03% of such kind *Pediococcus*. We observed the predominance of bacteria of the genus *Lactobacillus* throughout the fermentation. The measurements pH showed a decrease in pH of pimento during fermentation of 6.14 to 3.80. The *Lactococcus* or *Streptococcus* and *Pediococcus* appear after 24 hours of fermentation and disappear after 48 hours. The genus *Pediococcus* was absent in samples from some markets. Lactobacillus strains grow at pH ranging from 5 to 9. The growth depending on the temperature and the NaCl concentration of the bacteria isolated varies among species.

Introduction

The pepper (*Capsicum fructescens*) is a plant of the nightshade family. It is used as a condiment in food; it is consumed either raw green or ripe red or yellow depending on the type. However, in some cases it is often transformed (Jolicoeur, 2001; Cirad-gret, 2002; Caballero *et al.*, 2003; Coon, 2003). There are several spice processing techniques

that vary from one country to another, which leads to variable organoleptic quality. In Congo The technique is to grind the peppers and let them ferment in the bottles. Several lactic bacteria were isolated from the fermentation of plant substances.

These lactic acid bacteria ensure the preservation and improvement of the organoleptic qualities of the products obtained (Leveau and Bouix 1993). Thus, we were interested in the study of biodiversity of bacteria in fermented pepper and determine their physicochemical parameters.

Materials and Methods

The material consisted of samples of fermented peppers purchased in different markets of Brazzaville and a sample of fresh pimento and fermented in laboratory.

Sample preparation

The analyzed samples were prepared from a stock solution obtained homogéinisate 10 grams of peppers harvested every 24 hours of fermentation and 90 ml of diluents (Maximum recovery CMO733). Dilution of 10^{-1} to 10^{-6} were made from the stock solution.

Determination of pH

The sample pH was determined using pH meter microprocessor brand HANNA instruments HI 93321 every 24 hours of fermentation from a homogeinisat of 20 grams of pepper in 60ml of sterile distilled water.

Isolation purification and count Bacteria

The different dilutions were placed on medium Man Rogosa Sharpe (MRS; CM0361) on agar Petri dishes and incubated at 30 ° C for 24 to 48 hours. From the isolates, the different bacteria were purified and then counted.

Determination of physiological parameters

Physiological parameters were determined in a liquid medium in the tubes containing the Rogosa Sharpe broth (MRS; CM0359) at pH 6.2. 0.1 ml of each dilution was inoculated into 10 ml of broth Man Rogosa Sharpe.

The different bacteria was grown at pH 3.5; 5; 7 to 9. Growth temperatures were 37 ° C, 45 ° C and 55 ° C. Growth is followed with NaCl concentrations of: 2%, 4%, 6%, 8% and 10%. Search catalase and gas production were carried out by the method of Gibson and Abdel Malek.

Results and Discussion

Evolution of pH during Pepper fermentation

The different samples of pepper showed a progressive decrease in pH from 6.14 to 3.80 during fermentation as shown in Figure 1.

Evolution the microflora of pepper

The bacterial microflora pepper gradually increases. The amount of bacteria passes of 7.719 between 0 and 24 hours at 9875 between 72 and 96 hours.

The results are shown in Figure 2. Table 1 provides information on the evolution of various bacteria based on the fermentation time.

Frequency of isolated bacteria

The morphological and biochemical characteristics have given the following identification: *Lactobacillus* 28 strains (84.85%), 4 strains of *Streptococcus* (12.12%)

or a strain *Lactococcus and Pediococcus* (3.03%). The results are reported in figure 3 and Table 2.

Determination of physiological parameters of Pepper isolated bacteria

Monitoring the growth as a function of pH, temperature, the NaCl concentration and the results of catalase and those of the gas production of different bacteria are given in Tables 3 – 10.

The Fermentation of peppers is an important phenomenon for improving its organoleptic quality. Occurs mid acidification of the medium during the fermentation.

Studies of other fermented foods such as the Sauerkraut and the retted cassava dough have shown that acidification (Louembé *et al.*, 1998). This acidification is related to the presence of lactic bacteria of the genera: *Lactobacillus*, *Streptococcus or Lactococcus and Pediococcus* which are usually isolated from fermented food. These results are comparable to those given by (Yimin *et al.*, 1999, Adelfo *et al.*, 2001; Said Ennahar *et al.*, 2003, Vuyst *et al.*, Luke 2002, Myung *et al.*, 2005, Badis *et al.*, 2005).

The study of microflora shows a predominance of the genus Lactobacillus this result is consistent with that given by Yimin *et al.*, (1999).

On growth parameters, the results obtained on the Ph of growth are similar to those obtained by Louembé *et al.*, (2003) on fermented cassava leaves.

Table.1 Distribution of the main genera according to the fermentation places

Marquet Places Strains	**Market of Moungali**	**Market of Ouenze**	**Market of Total**	**Laboratory sample**
Pediococcus	**100%**	**0%**	**0%**	**0%**
Lactobacillus	10,71%	10,71%	21,43%	57,15%
Lactococcus* or *Streptococcus	**0%**	**0%**	**0%**	100%

Table.2 Evolution of different of lactic bacteria during the Peppers fermentation

	Time of fermentation				
Time	**0 hour**	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Genera of Bactriaisolated	*Lactobacillus*	*Streptococcus* or *Lactococcus*	*Streptococcus* or *Lactococcus*	*Streptococcus* or *Lactococcus*	***Lactobacillus***
	Lactobacillus	*Lactobacillus*	*Lactobacillus*	*Streptococcus ou Lactococcus*	***Lactobacillus***
	Lactobacillus	*Lactobacillus*	*Lactobacillus*	*Lactobacillus*	***Lactobacillus***
	Lactobacillus	***Lactobacillus***		***Lactobacillus***	***Lactobacillus***

Table.3 Growth of twenty eight (28) Lactobacillus strains isolated from pimento fermented at different pH

pH	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28
3,5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Legend: (+) growth
(-)= No growth

Table.4 Growth of *Lactobacillus* strains according to the salt concentration (NaCl)

NaCl (%)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
10	+	-	-	-	+	-	-	+	-	-	+	-	+	-	-	+	-	-	-	+	+	-	+	-	-	+	-	-

Legend: (+) growth
(-)= No growth

Table.5 Growth of *Lactobacillus* strains according to the temperature

T (°C)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28
30	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45	-	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
55	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Legend: (+) growth
(-)= No growth

Table.6 Catalase and gas production in *Lactobacillus* strains

Cultural characters	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28
Gaz	+	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+): Catalase positive and gas produce at bacteria
(-): no gas produce and catalase negative

Table.7 Growth of four (4) *Lactococcus* strains and one (1) *Pediococcus* strain at different pH

pH	3,5	5	7	9
L1	-	+	+	+
L2	-	+	+	+
L3	-	+	+	+
L4	-	+	+	+
P1	-	+	+	+

L : *Lactococcus* strains
P : Pediococcus strain

Table.8 Growth of four (4) *Lactococcus* (*Streptococcus*) strains and one (1) *Pediococcus* strain according to the salt concentration

NaCl (%)	2	4	6	8	10
L1	+	+	-	-	-
L2	-	+	+	-	-
L3	-	+	+	+	-
L4	-	+	+	+	-
P1	-	+	+	+	-

Table9 Growth of four (4) *Lactococcus* strains and one (1) *Pediococcus* strain at different temperatures

T¨° C	30	37	45	55
L1	+	+	+	-
L2	+	+	+	-
L3	+	+	+	-
L4	+	+	+	-
P1	+	+	+	-

Table.10 Catalase and gaz production in four (4) *Lactococcus* (*Streptococcus*) strains and one (1) *Pediococcus* strain

Strains	Gas Production	Catalase
L1	-	-
L2	-	-
L3	-	-
L4	-	-
P1	-	-

Fig.1 Evolution of pH during pepper fermentation

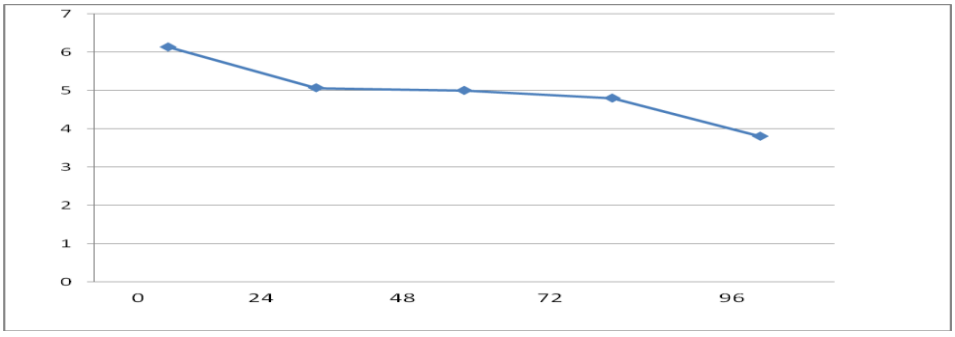


Fig.2 Evolution of the microflora

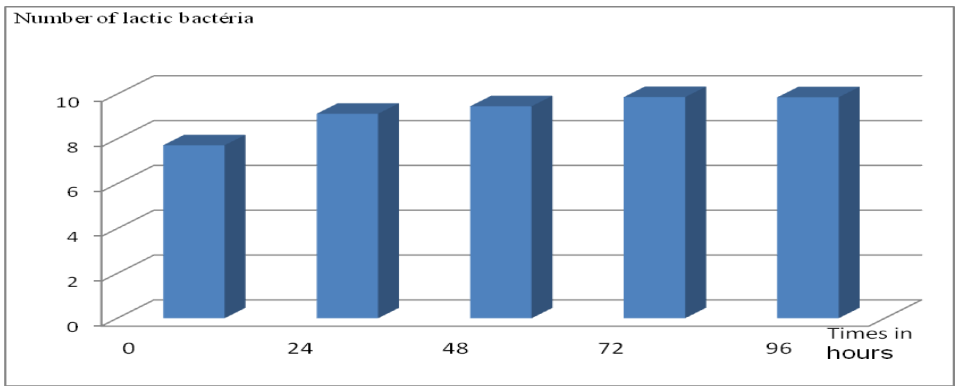
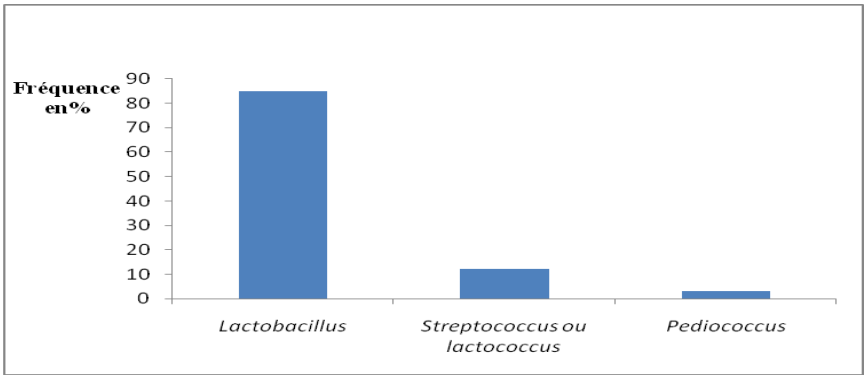


Fig.3 Frequency of isolated bacteria



As regards the growth temperature and the concentration of NaCl, the results are similar to those of Chen MM *et al.,* (2013).

In conclusion the peppers fermentation is the work of lactic bacteria of Genera *Lactobacillus, Streptococcus* and *Pediococus*. These bacteria tolerate PH, temperature and different concentrations of NaCl. their presence causes acidification of the medium. These bacteria have adopted different strategies to adapt to the various conditions for their survival in various environments. They change their metabolism according to different temperatures and developed abilities to grow at high NaCl concentrations, then the tolerance of acid or basic pH. These are bacteria having metabolic diversity can be harnessed for the industrial production of the spice.

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