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

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ABSTRACT

The pepper (Capsicum frutescens) 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 was made of 0.1ml seeded suspension on MRS CM0361 pH 6. 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.

Keywords
Biodiversity, Lactic bacteria, Physiological parameters, Food fermented

Introduction

The pepper (Capsicum frutescens) 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 homogèinisate 10 grams of peppers harvested every 24 hours of fermentation and 90 ml of diluents (Maximum recovery CM0733). 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 homogéinisat 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; CM0359) 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 | 0 hour | 24 hours | 48 hours | 72 hours | 96 hours |
|----------------------|--------|----------|----------|----------|----------|
| Genera of Bacteria isolated | *Lactobacillus* | *Streptococcus or Lactococcus* | *Streptococcus or Lactococcus* | *Streptococcus or Lactococcus* | *Lactobacillus* |
| *Lactobacillus*      | *Lactobacillus* | *Lactobacillus* | *Lactobacillus* | *Lactobacillus* | *Lactobacillus* |
| *Lactobacillus*      | *Lactobacillus* | *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 |
|---------------------|----|----|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 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       | -  | +  | +  | +  | -  |
Table 9: Growth of four (4) *Lactococcus* strains and one (1) *Pediococcus* strain at different temperatures

| Temperature °C | 30 | 37 | 45 | 55 |
|---------------|----|----|----|----|
| L1            | +  | +  | +  | -  |
| L2            | +  | +  | +  | -  |
| L3            | +  | +  | +  | -  |
| L4            | +  | +  | +  | -  |
| P1            | +  | +  | +  | -  |

Table 10: Catalase and gas 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
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 Pediococcus. 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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