Characterization of Lactobacillus Isolated from the Microflora of Different Fermentation Processes and Doderlein Flora

Coulibaly Adja Mansagna a,b*, Coulibaly Ibourahema a, Ouattara Mohamed Baguy b,c, Tiekoura Konan Bertin b,c, Guédé Kipre Bertin b,c, Toty Abale Anatole b,c, Konan Kouadio Fernique b,c, Mariko Safiatou Tenin. Soumahoro b,c, Nathalie Kouadio Guessennd b,c,d and Le GER-BMR c

a Laboratoire d’Agrovalorisation, Université Jean Lorougnon Guédé de Daloa, BP 150 Daloa, Côte d’Ivoire.
b Département de Bactériologie et Virologie, Institut Pasteur de Côte d’Ivoire, 01 BP 490 Abidjan 01, Côte d’Ivoire.
c Groupe d’Etude et de Recherche Sur les Bactéries Multi-Résistantes (GER-BMR), Institut Pasteur de Côte d’Ivoire, Côte d’Ivoire.
d Unité de Recherche et de Formation des Sciences Médicales, Université Felix Houphouët, Boigny, Abidjan, Côte d’Ivoire.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2023/v23i10756

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105973

Received: 05/07/2023
Accepted: 12/09/2023
Published: 18/09/2023

Original Research Article

*Corresponding author: E-mail: hadjamansagna@gmail.com;
ABSTRACT

*Lactobacillus* contribute to the organoleptic quality of foods during the fermentation process in which they are involved. They also have the ability to inhibit the growth of undesirable germs by producing metabolites such as bacteriocins. The aim of this study was to isolate and identify the different *Lactobacillus* species from various fermentation processes and from the Doderlein flora. *Lactobacillus* were isolated from raw cow milk, curdled milk, cassava ferment (Magnan) and Doderlein flora on MRS agar. Strains were identified by mass spectrometry (MALDI-TOF). 213 *Lactobacillus* strains were isolated and identified from the different matrices. The main species isolated from raw cow's milk and curdled milk were *Lactobacillus plantarum* (31.58%), *Lactobacillus casei* (26.31%), *Lactococcus lactis* (21.05%), *Lactobacillus delbrueckii* spp *bulgaricus* (31%), *Lactobacillus helveticus* (30%) and *Lactobacillus fermentum* (25%) respectively. In the cassava ferment, *Lactobacillus plantarum* (37.5%), *Lactobacillus rhamnosus* (25%) and *Lactobacillus reuteri* (25%) were the most isolated species. The main species of Doderlein flora were *Lactobacillus acidophilus* (50%) and *Lactobacillus plantarum* (25%). This study showed a diversity of *Lactobacillus* present and involved in the different fermentation processes of foods and Doderlein flora (Commensal flora of the vagina). The qualitative and quantitative proportions of the different *Lactobacillus* species depend on the matrix. These *Lactobacillus* can potentially produce substances (bacteriocins) that inhibit the growth of pathogenic bacteria and multi-resistant bacteria.

Keywords: *Lactobacillus*; biodiversity; fermented food; doderlein flora.

1. INTRODUCTION

*Lactobacillus* form a heterogeneous group of bacteria, ubiquitous and widespread in the plant, animal and human environment [1,2]. Bacteria of the genus *Lactobacillus* sp are Gram-positive, immobile, facultative anaerobic and homofermentative bacteria [3,4]. The species are numerous and relatively difficult to characterise and identify. Rarely pathogenic to humans, *Lactobacillus* probably also play a physiological role in humans and animals. They make a major contribution to many food preparations, particularly through lactic fermentation, in the production of cheeses, yoghurts and many other milk derivatives [2]. They are ubiquitous in many biotopes, such as fermented foods [5]. *Lactobacillus* are widely used in the food industry because of their fermenting power. They also contribute to the organoleptic quality of foodstuffs during the fermentation process, where they are involved [6]. Several studies have demonstrated the inhibitory power of lactic acid bacteria through the production of metabolites such as bacteriocins [7,8]. These bacteriocins have the ability to inhibit the growth of undesirable germs. In Ivory Coast, *Lactobacillus* are involved in several fermentation processes, such as the preparation of cassava ferment (mangna), the preparation of curdled milk and the fermentation of cocoa beans. *Lactobacillus* play a dual role in these fermentation processes. Firstly, they contribute to the final organoleptic quality of the food and, secondly, they inhibit the growth of pathogenic bacteria [9]. *Lactobacillus* are naturally present in the Doderlein flora (vaginal flora) where they play a protective role in the vaginal cavity against the proliferation of pathogenic germs [10-12]. The determination of the biodiversity of *Lactobacillus* in the various fermentation processes and in the Doderlein flora is more than necessary for studying their inhibiting power on multi-resistant bacteria.

The aim of this study is to identify the different *Lactobacillus* species involved in the main fermentation processes and the Döderlein flora, for the study of their inhibitory power.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 180 samples belonging to 3 food matrices (raw cow milk, curdled milk and manioc ferment) and to the Döderlein flora were taken in different localities in the city of Abidjan in Ivory Coast (Table 1). These localities were chosen because of their high production of these food matrices. Samples (vaginal swabs) of Döderlein flora were provided by the Genital Tract Agents Unit (GT-AU) of the Institut Pasteur de Côte d'Ivoire (IPCI).
Table 1. Distribution of the samples according to the different localities in Abidjan

<table>
<thead>
<tr>
<th>Location</th>
<th>Raw cow milk</th>
<th>Curdled milk</th>
<th>Cassava ferment</th>
<th>Döderlein flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>20</td>
<td>15</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>40</td>
<td>60</td>
<td>30</td>
</tr>
</tbody>
</table>

2.2 Enumeration of *Lactobacillus* sp in Food Matrices

For each matrix, 10 g (solid feed) or 10 ml (liquid feed) of the sample was added to 90 ml of buffered peptone water. The mixture was homogenized in a stomacher bag. This solution constituted the stock solution. From this stock solution, 1 mL was taken and mixed with 9 mL of buffered peptone water in a test tube. This gave the 10⁻¹ dilution. Then 1 ml of the previous dilution was added to 9 ml of buffered peptone water to obtain the next dilution. The other decimal dilutions were made up to 10⁻⁸. Petri dishes (2 dishes per dilution considered) were inoculated by spreading 0.1 µL of the inoculum on the surface of MRS agar. The Petri dishes were then incubated at 37°C in a jar under anaerobic conditions and at 44°C from 24 to 48 hours. For the bacterial counts, all Petri dishes containing at least 30 colonies and at most 300 colonies were selected. The results were expressed in Colony Forming Units (CFU) using the formula (NF ISO 7218/A1) [13].

\[ N = \frac{\sum C_i}{(N_1 + 0.1 N_2) V} \]

Where,

\( \sum C_i \): is the sum of the colonies counted on all the plates retained from two successive dilutions

\( V \): the volume of inoculum applied to each dish

\( N_1 \): the number of plates retained for the first dilution considered

\( N_2 \): the number of boxes retained for the second dilution considered

\( D \): first dilution considered

2.3 Morphological Identification of *Lactobacillus* sp

On the Petri dishes (10⁻⁴ to 10⁻⁷) used for counting, five colonies chosen at random on the basis of their macroscopic appearance on MRS agar were identified. Gram-positive bacilli or cocci colonies, catalase and oxidase negative were selected for further work [14]. Presumptive colonies of *Lactobacillus* sp were stored at 20°C in Eppendorf tubes containing Brain Heart Broth (BCC) plus glycerol [3].

2.4 Identification by Mass Spectrometry (MALDI-TOF)

To carry out this test, the colony of *Lactobacillus* sp in subculture for 24 hours was removed using a 10 µl calibrated loop and then placed on the plate. Each plate can be used to identify 24 strains. A matrix (α-Cyano-4-hydroxycinnamic acid) was used to cover each spot. The operation was carried out using the reference strain of *Escherichia coli* DH5 alpha, which was used as a quality control for the MALDI-TOF identification manipulation. The plate was then inserted into the mass spectrometer. Results were analyzed on the computer workstation (laboratory computer system) associated with the spectrometer [15].

3. RESULTS

3.1 Enumeration of *Lactobacillus* sp

The enumeration results observed according to the different temperatures ranged from 0.549. 10⁷ CFU/ml to 11. 10⁷ CFU/ml. They are reported in Table 2. Enumeration shows that the average number of *Lactobacillus* strains varies from 5.110⁶ CFU/ml to 1.67.110⁷ CFU/ml in the Anyama commune and 2.98. 10⁷ CFU/ml in Yopougon (Adiopodoumé); in samples of raw cow's milk; in cassava ferments for the two communes the average number of *Lactobacillus* strains varies between 3.10⁶ CFU/ml and 1.5. 10⁷ CFU/ml; in curdled milk samples (Adjame and Abobo), the number varies between 1.9. 10⁷ CFU/ml and 11. 10⁷ CFU/ml (Table 2).

3.2 Isolation of *Lactobacillus* sp.

A total of 213 strains of *Lactobacillus* sp were isolated on MRS (fig.1) from different matrices

- 60 strains from raw cow milk,
- 57 strains from curdled milk,
- 63 strains from manioc ferments
- 33 strains from human Döderlein flora. All were selected for identification by MALDI-TOF.
Table 2. Average number of colonies in CFU/ml observed by matrix for each commune

<table>
<thead>
<tr>
<th>Location</th>
<th>Anyama</th>
<th>Adiopodoumé</th>
<th>Cocody</th>
<th>Ebimpé</th>
<th>Adjame</th>
<th>Abobo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw cow milk</td>
<td>1.67x10^7</td>
<td>2.98x10^7</td>
<td>1.5x10^7</td>
<td>0.875x10^7</td>
<td>1.9x10^7</td>
<td>9x10^7</td>
</tr>
<tr>
<td>Cassava ferments</td>
<td>5.1x10^6</td>
<td>3.1x10^6</td>
<td>3.1x10^6</td>
<td>7.123x10^7</td>
<td>11x10^7</td>
<td></td>
</tr>
<tr>
<td>Curdled milk</td>
<td>5.1x10^6</td>
<td>3.1x10^6</td>
<td>3.1x10^6</td>
<td>7.123x10^7</td>
<td>11x10^7</td>
<td></td>
</tr>
</tbody>
</table>

3.3 Morphological Characterization (GRAM Staining)

Microscopic observation after Gram staining identified two forms: shells and rods. The rod-shaped forms represent 90% of the total number of bacteria and are represented by Lactobacillus sp strains (all Gram-positive, immobile, catalase-negative bacilli). The shells observed make up the remaining 10% (Fig. 2).

3.4 Identification by Mass Spectrometry (MALDI-TOF)

Identification by MALDI-TOF identified a total of 10 Lactobacillus species: Lactobacillus plantarum, Lactobacillus casei, Lactobacillus delbrueckii spp bulgaricus, Lactobacillus helveticus, Lactobacillus fermentum, Lactobacillus rhamnosus and Lactobacillus reuteri, Lactobacillus paracasei, Lactobacillus acidophilus, Lactobacillus bulgaricus isolated from four matrices (raw cow milk, curdled milk, cassava ferment and human Döderlein flora).

Seven Lactobacillus species were identified from raw cow’s milk. Among these, Lactobacillus plantarum was the most dominant species with a rate of 37.25%, followed by Lactobacillus casei with a rate of 31.5%. On the other hand, the species Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus reuteri and Lactococcus lactis were isolated very little, with proportions between 6.25 and 2.5% (Table 3).
The identification results revealed five (5) \textit{Lactobacillus} species in the curdled milk (Table 4). Among the different species identified, \textit{Lactobacillus fermentum} and \textit{Lactobacillus bulgaricus} were the most isolated with proportions of 45.45 and 24.2\% respectively. Very few \textit{Lactobacillus plantarum} species were isolated (6.1\%) (Table 4).

Four \textit{Lactobacillus} species isolated from cassava ferments were identified (Table 5). It should be noted that \textit{Lactobacillus plantarum} was the most dominant species with a rate of 46.7\%. The \textit{Lactobacillus bulgaricus} species was the least dominant, with a low rate of 6.7\%. It should also be noted that the \textit{Lactobacillus plantarum} species was isolated to a lesser extent with a rate of 23.3\% (Table 6).

### 4. DISCUSSION

The results of identification by mass spectrometry (MALDI-TOF) showed a diversity of \textit{Lactobacillus} species isolated from raw cow's milk, curdled milk, Döderlein flora and cassava ferments.

Seven species of \textit{Lactobacillus} (\textit{Lactococcus lactis}, \textit{Lactobacillus acidophilus}, \textit{Lactobacillus rhamnosus}, \textit{Lactobacillus reuteri}, \textit{Lactobacillus plantarum}, \textit{Lactobacillus casei}, \textit{Lactobacillus paracasei}) isolated from raw cow milk were identified using the MALDI-TOF technique. Our results are similar to those reported by [16,17] who identified 5 of our \textit{Lactobacillus} species namely \textit{Lactobacillus casei}, \textit{Lactobacillus paracasei}, \textit{Lactobacillus plantarum} \textit{Lactobacillus rhamnosus} and \textit{Lactococcus lactis}. These species have been identified in raw cow's milk and camel's milk. The wide diversity of \textit{Lactobacillus} species isolated from our samples

### Table 3. Identification of \textit{Lactobacillus} species isolated from raw cow milk

<table>
<thead>
<tr>
<th>\textit{Lactobacillus} species</th>
<th>La. lactis</th>
<th>Lb. acidophilus</th>
<th>Lb. rhamnosus</th>
<th>Lb. reuteri</th>
<th>Lb. plantarum</th>
<th>Lb. casei</th>
<th>Lb. paracasei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of strains</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>30</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Percentage</td>
<td>2.5 %</td>
<td>6.25 %</td>
<td>6.25 %</td>
<td>6.25 %</td>
<td>37.25 %</td>
<td>31.25 %</td>
<td>10 %</td>
</tr>
</tbody>
</table>

La. : \textit{Lactococcus}; Lb. : \textit{Lactobacillus}

### Table 4. Identification of \textit{Lactobacillus} species isolated from curdled milk

<table>
<thead>
<tr>
<th>\textit{Lactobacillus} species</th>
<th>Lb. bulgaricus</th>
<th>Lb. helveticus</th>
<th>Lb. fermentum</th>
<th>Lb. plantarum</th>
<th>Lb. casei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of strains</td>
<td>8</td>
<td>3</td>
<td>15</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Percentage</td>
<td>24.2%</td>
<td>9.1%</td>
<td>45.45%</td>
<td>6.1%</td>
<td>15.15%</td>
</tr>
</tbody>
</table>

Lb. : \textit{Lactobacillus}

### Table 5. Identification of \textit{Lactobacillus} species isolated from cassava ferment

<table>
<thead>
<tr>
<th>\textit{Lactobacillus} species</th>
<th>Lb. rhamnosus</th>
<th>Lb. reuteri</th>
<th>Lb. casei</th>
<th>Lb. plantarum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of strains</td>
<td>15</td>
<td>15</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Percentage</td>
<td>21.43 %</td>
<td>21.43 %</td>
<td>7.15 %</td>
<td>50 %</td>
</tr>
</tbody>
</table>

Lb. : \textit{Lactobacillus}

### Table 6. Identification of \textit{Lactobacillus} species isolated from the Döderlein flora

<table>
<thead>
<tr>
<th>\textit{Lactobacillus} species</th>
<th>Lb. acidophilus</th>
<th>Lb. plantarum</th>
<th>Lb. bulgaricus</th>
<th>Lb. paracasei</th>
<th>Lb. fermentum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of strains</td>
<td>14</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Percentage</td>
<td>46.7 %</td>
<td>23.3 %</td>
<td>6.7 %</td>
<td>10 %</td>
<td>13 %</td>
</tr>
</tbody>
</table>

Lb. : \textit{Lactobacillus}
could be explained by the different methods used to identify the different species. According to the results obtained in our experiment, we also agree with those reported by [18] who identified by MALDI-TOF and PCR the same Lactobacillus species (Lactobacillus bulgaricus, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus acidophilus and Lactobacillus casei) isolated from cassava and the acid pH [28]. Our results are due to the effect of the lactose present in fermented milk. The diversity of species involved in the various food fermentation processes (raw cow milk, curdled milk and cassava ferment) and present in the Döderlein flora. This diversity could be an asset, increasing the chances of finding species that produce inhibiting substances (bacteriocins). The bacteriocins produced by these species could contribute to the fight against pathogenic bacteria and multi-resistant bacteria.

Our results are practically similar to those reported by [29] who concluded that the Lactobacillus most frequently isolated from healthy vaginas are in approximate proportions. These Lactobacillus are Lactobacillus fermentum (1.1%), Lactobacillus crispatus (48.3%), Lactobacillus jensenii (25.3%), Lactobacillus gasseri (23.5%), Lactobacillus iners (20.5%), Lactobacillus vaginalis (11.6%), Lactobacillus reuteri (1.4%), Lactobacillus rhamnosus (0.9%).

Among the Lactobacillus species isolated in our samples of the commensal flora of the vagina, Lactobacillus acidophilus is the most dominant species with a rate of 46.7%. Our results are similar to those reported by [30,31] who also found this species to be dominant in Chinese pregnant women. Our results are also in agreement with those reported by [32,33], who identified several Lactobacillus species in the vagina of women with normal flora, including Lactobacillus acidophilus.

5. CONCLUSION

This study enabled us to quantitatively and qualitatively identify several Lactobacillus species involved in the various food fermentation processes (raw cow milk, curdled milk and cassava ferment) and present in the Döderlein flora. This diversity could be an asset, increasing the chances of finding species that produce inhibiting substances (bacteriocins). The bacteriocins produced by these species could contribute to the fight against pathogenic bacteria and multi-resistant bacteria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

5. Hammi I. Isolation and characterization of bacteriocins produced by strains of lactic acid bacteria isolated from Moroccan fermented products and different varieties of French cheeses. Doctoral thesis in Analytical Chemistry, Faculty of Pharmacy, Université Strasbourg. 2016;150.


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Peer-review history: The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/105973