Biodiversity, dynamics and antimicrobial activity of lactic acid bacteria involved in the fermentation of maize flour for doklu production in Côte d'Ivoire

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Abstract
Doklu is a maize-based spontaneously fermented dough produced and consumed in parts of West Africa, particularly in Côte d'Ivoire. The characterization of the microbial ecosystem of doklu was carried out using a polyphasic approach. First, culture-dependent methods were used for bacterial enumeration and the phenotypic and molecular identification of 250 lactic acid bacteria (LAB) isolates. Then, culture-independent methods, including PCR-TTGE (V3 region of the 16S rRNA gene), provided a fingerprinting of bacterial DNA directly extracted from doklu. Bio preservative abilities were also tested and strains producing antimicrobial compounds were genotyped using PFGE. During maize dough fermentation, LAB became dominant and their load increased from 4.2 ± 0.2 log CFU/g to 9.0 ± 0.7 log CFU/g only after 48 h. Culture-dependent methods highlighted the presence of five LAB groups with the species Lactobacillus plantarum (28%), Lactobacillus fermentum (41.6%), Pediococcus acidilactici (6.8%), Pediococcus pentosaceus (18%) et Weissella cibaria (5.6%), succeeding during the fermentation. Lb. fermentum being practically the only species present at the end of fermentation, is with Lb. plantarum, the predominant species of fermenting dough. Culture-independent analysis underlined the undoubted role of Lb. fermentum, actively involved in the dough fermentation. These Lb. fermentum species, with a diversity of strains also showed important antimicrobial activity, due to production of bacteriocins. Being able to produce antimicrobial compounds, Lb. fermentum species may act as both bio protective culture as well as fermenting agent in cereal products and could be exploited to create functional starter cultures.

1. Introduction
Fermentation of cereals, such as millet (Pennisetum glaucum), maize (Zea mays) and sorghum (Sorghum bicolor), to produce gruel, dough and porridge is very popular throughout Africa, where they are consumed as complementary food for young children, adult main meals and beverages. Ogi and kunu-zaki in Nigeria, gowê in Benin, koko or akasa and kenkey in Ghana, ben-sanliga in Burkina Faso, bozu in South Africa, pototopo in Congo and doklu in Côte d'Ivoire are all examples of traditional cereal foods produced by uncontrolled natural fermentation with occasional usage of back slopping techniques to initiate fermentation (Assohoun, Djéni, N’Guessan, & Koussemon, 2012; Halm, Amoa-Awua, & Jakobsen, 2004; Tou et al., 2006; Vieira-Dalode et al., 2007). In particular for doklu, processing involves cleaning and washing of whole maize grains, 1 or 2 days water soaking of cereal, wet milling, 2–3 days fermentation at ambient temperature, wrapping in maize husks and cooking (Assohoun et al., 2012). It is one of the various traditional fermented foods of Côte d’Ivoire, produced mainly in the southern parts of the country at household level and for family consumption only.
The people often appreciate doko for its sour taste due to fermentation. Indeed, doko production like many other indigenous fermented foods traditionally relies on spontaneous fermentation initiated by natural microorganisms that are found on raw materials/ingredients, on the processing utensils/equipments, on fermented foods traditionally relies on spontaneous fermentation.

2. Materials and methods

2.1. Sample collection

The different samples (250 g) for this study were collected in sterile containers from six processors at different stages of processing (maize grains, milled flours, fermented dough and finished product doko) in Abidjan (the economic capital of Côte d’Ivoire). All collected samples were immediately transported in an icebox directly to the laboratory for analyses.

2.2. Enumeration of microorganisms

Preparation of stock solutions, inoculation of agar plates, and cultivation and quantification of microorganisms were carried out according to Coulin, Farah, Assanvo, Spillman, and Puhun (2006). For all determinations, 10 g of the sample were homogenized in a stomacher with 90 ml of sterile diluent containing 0.85% NaCl and 0.1% peptone (Difco, Becton Dickinson, Sparks, MD, USA). Ten-fold serial dilutions of stomacher fluid, ranging from 10^1 to 10^7, were prepared and spread-plated for the determination of microbrial counts. So, enumeration of coliforms was carried out using VRBL (Violet crystal and neutral Red Bile Lactose) plates containing agar (VRBL agar, Oxoid Ltd., Basingstore, UK), incubated for 24 h at 30 °C for total coliforms and 44 °C for fecal coliforms. Yeasts and molds were enumerated on Sabouraud chloramphenicol agar (Fluka, Biochemica 89579, Sigma–Aldrich Chimie GmbH, India) incubated at 30 °C for 4 days. Aerobic mesophiles were enumerated on Plate Count Agar (PCA Oxoid) and incubated at 30 °C for 2 days. Enumeration of LAB was carried out using Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany), which were incubated under anaerobic conditions (Anaerocult A, Merck) at 37 °C for 72 h.

2.3. Identification of LAB isolates

Isolation of LAB was performed from fermenting maize dough samples as follows: about 5 colonies were randomly selected among 20 by picking colonies from plates of highest dilutions showing growth. Collected isolates (250) were purified twice on MRS agar. Each isolate was then characterized for Gram reaction, catalase activity by the 3% H2O2 method and cytochrome oxidase production by Bactident Oxidase reagent (Merck). Then, the chromosomal DNA of the 250 isolates was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, S.A., Courtaboeuf, France). Identification of isolates was performed by determining the 16S rDNA gene sequence. The respective 16S rDNA genes were first amplified using the primers 1d1 and 1d1 (Table 1) and a previously described PCR program (Weisburg, Barms, Pelletier, & Lane, 1991). The resulting sequences were assembled into a unique contig with BioEdit sequence alignment software and then submitted to the NCBI database (NCBI, Bethesda, USA, http://www.ncbi.nlm.nih.gov/) for representation of sequence and similarity searches in the GenBank database.

2.4. TTGE analysis

The chromosomal DNA of the 5 LAB references strains (Lactobacillus plantarum ATCC 14917, Lactobacillus fermentum DSM 20052, Pedococcus acidilactici CIP 103408T, Pedococcus pentosaceus CIP 102260 and Weissella cibaria DSM 15878) and bacterial DNA from the fermenting maize dough matrix extracted following the entire protocol described by Macé et al. (2012), were analyzed by PCR-TTGE. Primers V3P2 and V3P3 (Table 1) were used to amplify V3 region (194 bp) PCR-amplicons as previously described (Jaffrès et al., 2009). The size of the PCR products was determined in a 1.5% (w/v) agarose gel (Invitrogen) using an exACTGene 100 bp PCR DNA Ladder (Fisher Scientific, Illkirch, France).

TTGE gel analysis was performed using the DCode universal mutation detection system (BioRad, Marne-la-Croquette, France) according to the method of Macé et al. (2012). The electrophoresis run was performed at 50 V for 12 h 30 min with a temperature gradient of 65 °C–70 °C (rate of 0.4 °C h⁻¹) under stirring with a magnetic stirrer to mix the buffer and improve the temperature gradient homogeneity (Ogier, Son, Gruss, Tailliez, & Delacroix-Buchet, 2002).

The PCR products (of the 5 reference strains) were moved at the same time with fermenting maize dough samples. The repro
2.5. Antimicrobial activity of LAB isolates

2.5.1. Microorganisms, culture media and growth conditions

LAB strains (250) isolated from fermenting maize dough and identified by whole 16S RNA gene sequencing were grown twice at 37 °C in MRS broth and Wheat flour hydrolysate (WFH) (Gobbetti, Corsetti, & Rossi, 1994), for 24 h and 18 h, respectively. WFH used for determination of conidia germination was chosen as the substrate since representative of the chemical composition of wheat flour.

Table 1

<table>
<thead>
<tr>
<th>Primers</th>
<th>Position</th>
<th>Oligonucleotide sequence (5′ – 3′)</th>
<th>T° (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fd1</td>
<td>16S rDNA gene, F (positions 8-27)</td>
<td>AGAGTTTGATCCTGCGGCTAGG</td>
<td>56</td>
<td>(Weisburg et al., 1991)</td>
</tr>
<tr>
<td>rd1</td>
<td>16S rDNA gene, F (positions 1521-1542)</td>
<td>TAAGGCGAGTATCCGAGC</td>
<td>56</td>
<td>(Weisburg et al., 1991)</td>
</tr>
<tr>
<td>V3P1</td>
<td>16S rDNA gene, F (positions 317-333)</td>
<td>ATTACCGCGGGCGGATTCTG</td>
<td>62</td>
<td>(Parayre et al., 2007)</td>
</tr>
<tr>
<td>V3P2</td>
<td>16S rDNA gene, F (positions 340-356)</td>
<td>CCACCCCCCCCCCCCCCGGCGGGGCGGGG.CGCCCAGCCGGGCTACGGGAGGCAGCAG</td>
<td>62</td>
<td>(Parayre et al., 2007)</td>
</tr>
</tbody>
</table>

(*) Annealing temperature. F: forward.


ducibility and the specificity of species-specific TTGE profiles were confirmed by comparison of TTGE profiles of two or three strains of each species.


ducted antibacterial activity of LAB isolates

2.5.2. Assays of antifungal activity

The capacity of LAB isolates to inhibit growth of fungi was assayed according to the method of Codia et al. (2011), but using the agar diffusion assay in micro-plates and with different test media: modified MRS medium (mMRS) (1% Yeast extract, 1.5% sucrose, glucose, fructose and maltose, 20 mg/l bromocresol green, 7 g/l agar, pH 5.6) and WFH medium. So the antifungal activity is characterized following the indicator strain growth.

2.5.3. Assays of antibacterial activity

Antibacterial activities of LAB isolates were studied using the agar-well diffusion assay as described by Todorov and Dicks (2005) and Diop et al. (2008). LAB strains were grown twice in MRS broth, for 24 h at 37 °C and for 18 h at 37 °C. The broth (10 ml) obtained after incubation was centrifuged at 12,000 g for 10 min in a Hermle ZK236 (Hermle GmbH and Co, Gosheim, Germany) centrifuge. LAB cell-free supernatants obtained were adjusted to pH 6.8 with 1 M NaOH to eliminate organic acid effect, and filtered through a 0.22 μm (Millipore, Bedford, MA).

The antibacterial activity of LAB strains was shown by appearance of inhibition zones around wells. The diameter of the inhibition zone was measured (average of two perpendicular diameters) and results expressed in mm, including well diameters.

2.5.4. Proteolysis and heat stability of antimicrobial compounds

Neutralized supernatants (pH 6.8) of fresh LAB cultures were treated with catalase (5 mg/ml) (Merck, Dijon, France) and chymotrypsin (0.2 mg/ml) (Boehringer, Mannheim, Germany) for 1 h at 37 °C. Heat stability was determined by heating supernatants at 50 °C, 60 °C, 70 °C and 80 °C for 10 min. After treatments, the residual activity was determined by agar diffusion assay.

2.5.5. Pulsed field gel electrophoresis analysis of LAB species producing bacteriocins

Total DNA from LAB producing bacteriocin and belonging to the same species was analyzed by pulsed field gel electrophoresis (PFGE). The DNA was prepared in agarose plugs following the procedures of Coeuret, Guéguen, and Vernoux (2004). PFGE gels were visualized by UV transillumination at 254 nm and photographed with a KODAK DC290 zoom digital camera. The patterns were normalized and further processed with GelCompar version 2.5 (Applied Maths, Kortrijk, Belgium) (Vauterin & Vauterin, 1992). The similarities between the patterns are calculated with the Pearson coefficient using the BioNumerics software, version 7.1 (Applied Maths, 1998 to 2013, Kortrijk, Belgium). This software was also used to construct a dendrogram using the UPGMA (Unweighted Pair Group Method using Arithmetical Averages) algorithm.

2.6. Statistical analysis

All trials were repeated four times. The different sample treatments were compared by performing one-way analysis of variance on the replicates at a 95% level of significance using the Statistica (99th Ed, Alabama, USA) statistical program. Unless otherwise stated, significant results refer to P < 0.05. This software was also used to calculate mean values and standard deviations of the trials.

3. Results

3.1. Enumeration of the different bacterial groups

Enumeration of microorganisms in this study showed that LAB and yeasts and molds had similar growth throughout the fermentation but their counts at the different sampling steps were significantly different (P < 0.05) (Fig. 1). Indeed, in the dry maize grains, yeasts and molds mean load was 4.9 ± 0.3 log CFU/g against 4.2 ± 0.2 log CFU/g for LAB. These counts increased during the process and reached highest values (7.8 ± 0.1 log CFU/g for yeasts and molds and 9 ± 0.7 log CFU/g for LAB) in maize flour (obtained after milling grains previously soaked during 48 h in water), before decreasing until the end of fermentation (Fig. 1). However, the LAB count stayed significantly higher than that of yeasts and molds. Contrarily to the fermenting microorganisms (LAB and yeasts and molds), the total and fecal coliform populations, with respective initial loads of 4.2 ± 0.2 log CFU/g and 4.2 ± 0.21 log CFU/g decreased rapidly and disappeared only after 24 h of fermentation.
for fecal coliforms and 48 h for total coliforms. However, the aerobic mesophiles count increased from 5.8 ± 0.2 log CFU/g in grains to its highest values of 9.39 ± 0.1 log CFU/g in the flour, before decreasing during fermentation to 8.11 ± 0.1 log CFU/g (Fig. 2).

3.2. Identification of bacterial isolates

A total number of 250 isolates positive for Gram staining and negative for catalase and oxidase isolated on MRS agar were considered as LAB and identified after amplification and partial or total sequencing of their 16S rRNA gene, using the NCBI GenBank database. As seen in Table 2, the identification allowed to classify the 250 LAB isolates into 5 species namely Lb. fermentum, Lb. plantarum, P. pentosaceus, P. acidilactici and W. cibaria. The distribution of LAB species isolated from different samples shows the dominance of Lb. fermentum during the fermentation of maize to produce doklu (Table 2). In fact, average numbers of Lb. fermentum was significantly higher than all the other species identified throughout the fermentation. Lb. fermentum (group I) represented 41.6% of isolates; Lb. plantarum (group II), P. pentosaceus (group III), P. acidilactici (group IV) and W. cibaria (group V) represented 28%, 18%, 6.8% and 5.6% of the isolates, respectively.

The fermentation of maize dough during doklu processing was additionally characterized by a microbial succession. The initial stage of the fermentation was characterized by codominance of P. pentosaceus, P. acidilactici and Lb. fermentum, which represented 40%, 27% and 33% of isolates, respectively.

After 24 h of the fermentation, W. cibaria (16%) appeared with Lb. plantarum who became the main species (47%). P. pentosaceus species disappeared and a decrease in P. acidilactici number was observed. Lb. plantarum stayed the main species (56%) of LAB identified also after 48 h of fermentation. P. pentosaceus (39%) species appeared and the number of Lb. fermentum decreased to 5%. At the end of fermentation, Lb. fermentum was the only species found in maize dough (Fig. 3).

3.3. TTGE analysis

TTGE analysis enabled the dynamics of the microbiota to be visualized by examining fingerprints of LAB evolving during fermentation of maize dough (Fig. 4). In order to analyze the TTGE profiles and to detect the presence of LAB species, fingerprints of the different samples were compared with those of pure strains used as reference strains: W. cibaria DSM 15878, P. pentosaceus CIP
Table 2
Biodiversity of LAB identified based on BLAST comparison in GenBank, of the sequencing of the 16S rRNA gene.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No (%) of identified LAB</th>
<th>Number of nucleotides</th>
<th>Percentage of homology</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>104 (41.6)</td>
<td>720</td>
<td>99</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td>II</td>
<td>70 (28)</td>
<td>718</td>
<td>99</td>
<td>Lactobacillus plantarum</td>
</tr>
<tr>
<td>III</td>
<td>45 (18)</td>
<td>722</td>
<td>99</td>
<td>Pediococcus pentosaceus</td>
</tr>
<tr>
<td>IV</td>
<td>17 (6.8)</td>
<td>700</td>
<td>99</td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td>V</td>
<td>14 (5.6)</td>
<td>715</td>
<td>99</td>
<td>Weissella cibaria</td>
</tr>
</tbody>
</table>

* Number in brackets is percent of identified LAB.

Table 3
LAB identified by PCR-TTGE analysis at different fermentation times of maize dough for doklu production.

<table>
<thead>
<tr>
<th>Fermentation times (h)</th>
<th>No of identified species</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pediococcus pentosaceus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td>24</td>
<td>4</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pediococcus pentosaceus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pediococcus acidilactici</td>
</tr>
<tr>
<td>48</td>
<td>2</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pediococcus pentosaceus</td>
</tr>
<tr>
<td>72</td>
<td>2</td>
<td>Lactobacillus fermentum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pediococcus pentosaceus</td>
</tr>
</tbody>
</table>

Table 4
Inhibitory spectra of lactic acid bacteria isolated from fermenting maize dough for doklu production against five fungi strains on two culture media (mMRS and WFH).

<table>
<thead>
<tr>
<th>Fungi strains</th>
<th>Inhibition rates</th>
<th>Country of isolation</th>
<th>mMRS</th>
<th>WFH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eurotium repens</td>
<td>13 (81.25)</td>
<td>France</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Penicillium corylophilium</td>
<td>5 (31.25)</td>
<td></td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>5 (31.25)</td>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Wallemia sebi</td>
<td>13 (81.25)</td>
<td></td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Cladosporium sphaerospermum</td>
<td>13 (81.25)</td>
<td>A</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>

Inhibitory activity was determined by hphgal growth rates of fungi after 5 days of incubation at 25 °C. The results are expressed as number (percentage) of LAB with inhibitory activity among the 16 strains with at least one inhibitory activity.

* Only 16 out of the 250 LAB strains demonstrated antifungal activity.

4. Discussion

This study has shown the presence of LAB and yeasts and molds, in dry maize grains, maize flour and fermenting maize dough used for doklu production. These microorganisms have certainly contaminated maize grains during storage. They had been previously isolated in fermented food. Escalante, Wacher, and Farre (2001) reported their presence in pozol, a Mexican fermented maize dough. During fermentation of maize dough for doklu production, number of microorganisms increases highly during the first steps before decreasing slightly after 48 h. The abundance of these microorganisms during fermentation is due to their tolerance to acidic conditions. They commonly play very important roles in the production of a range of traditional maize and cereal-based fermented foods and beverages, including ogi (Odunfa & Adeyeye, 1985), kenkey (Hayford, Petersen, Vogensen, & Jakobsen, 1999), ogwa (Mugula, Nko, Narhus, & Sorhaug, 2003), koko (Lei & Jakobsen, 2004) and gowé (Vieira-Dalode et al., 2007) and have also previously been reported to be present in high numbers in fermented dough for doklu production (Assohoun et al., 2012). It has also been reported that LAB that survive the fermentation processes usually do this in association with yeasts (Sanni, 1993). In-
deed, these microorganisms (yeasts and LAB) are common in traditional fermented foods (Nyanga et al., 2007). A co-metabolism between these microorganisms has been suggested by Gobbetti et al. (1994). During this co-metabolism, LAB provide an acidic environment, which encourages the growth of yeasts, and yeasts provide vitamins and other growth factors for LAB. The decrease of these fermentative microorganisms at the end of the fermentation was certainly due to the reduction of nutrients in the maize dough. Other microorganisms (total coliforms, fecal coliforms and aerobic mesophiles) were also present in samples analyzed in this study. The main source of these undesirable bacteria has been reported to be the grinding process and minor numbers of them could have also been introduced during unhygienic handling of the dough. Similar observations were reported by Wacher, Canas, Cook, Barzana, and Owens (1993) by determining sources of microorganisms in pozol. But coliforms decreased rapidly and disappeared only after 24 h for fecal coliforms and 48 h for total coliforms. This disappearing of coliforms was certainly due to the sensitivity of these bacteria to the substances produced by LAB. It was proved that LAB exert antimicrobial action through the production of lactic and acetic acids, bacteriocins, diacetyl and hydrogen peroxide (Daeschel, 1989).

Moreover, this study was also achieved to highlight the biodiversity and dynamics of LAB communities involved in the fermentation of maize dough during doklu production. For this purpose, culture-dependent and culture-independent methods were combined to obtain a complete picture of the biodiversity of the maize dough ecosystem. This approach (16S rRNA sequencing and PCR-TTGE) was already used to study microorganism’s communities in several foods particularly in sourdough (De Vuyst & Van canneyt, 2007), cheese (Dolci et al, 2010) and over cereals-based foods. Using an initial grouping of LAB by phenotypic characteristics followed by identification by sequencing of the 16S rRNA gene, the dominant LAB species from six doklu production sites in southern Côte d’Ivoire were found to be Lb. plantarum, Lb. fermentum, P. acidilactici, P. pentosaceus and W. cibaria. Both methods (16S rRNA sequencing and PCR-TTGE) applied in this study lead to the same results, except for Lb. plantarum, which has not been identified by PCR-TTGE. These different LAB species identified in maize dough for doklu production were previously isolated in several foods such as sorghum and other fermented cereals-based foods (Olsen, Halm, & Jacobsen, 1995). During doklu processing, Lb. fermentum was present in high number at every stage of fermentation, confirming previous findings, which have revealed the predominance of Lb. fermentum in many natural lactic fermentations of plant materials (Nielsen et al., 2007; Sawadogo-Lingani et al., 2007). The role of Lb. fermentum in aroma formation has been described for fermented maize dough (Annan, Poll, Sefa-Deddeh, Plahar, & Jacobsen, 2003). The second predominant species after Lb. fermentum during doklu processing was Lb. plantarum. Kunene, Geornaras, Von Holy & Hastings (2000) showed that Lb. plantarum and Lb. fermentum were good associates in spontaneous fermentation of cereals-based foods. W. cibaria has been isolated in the fewest number and only after 24 h of fermentation. This species or other species of the same genus has been associated with some traditional millet-based fermented foods such as koko in Ghana (Lei & Jakobsen, 2004) and togwa in Tanzania (Mugula et al., 2003). During this study, no band corresponding to the reference strain Lb. plantarum ATCC 14917 was obtained in the TTGE profile. This could be due to the fact that fermenting maize dough contained a low DNA concentration from this species. The evaluation of TTGE sensitivity showed that the detection of species might be limited either by low DNA concentration or by the presence of a high concentration of competing DNA. Differences in the relative abundance of community members may affect the detection of certain species due to competition during PCR. Thus, the application of TTGE to detect minor species would require the use of highly specific primers (Ogier et al., 2002; Walter et al., 2000). Moreover, we observed distinct double band pattern derived from W. cibaria DSM 15878 and P. acidilactici CIP 103408T. Indeed, one drawback of the use of RNA coding genes is the inherent sequence heterogeneity within the same species, which is the result of multi-copies of the genes with small differences in the sequence. These multi-copies often result in multi-signals, which complicate the analysis. The rpoB gene, encoding for the β-subunit of the RNA polymerase, has been proposed as an alternative, but its application is still limited (Dahllof, Baillie, & Kjelleberg, 2000).

Moreover, another aim of the current study was to isolate and identify bacteriocin-producing LAB from the food that could be
used locally for improving food preservation (bio preservation) and biosafety. Sixteen isolates among the 250 LAB strains tested belonging to various species mainly Lb. fermentum, Lb. plantarum, P. acidilactici, P. pentosaceus and W. cibaria inhibited the growth of at least one of the five tested fungal strains. However, antibacterial activity was effective only against Lb. delbrueckii F31 and this activity was observed only for 20 Lb. fermentum strains, representing 8% of the tested strains, with inhibition diameters ranging from 11 to 20 mm. The antibacterial compounds were resistant to catalase and heat, but were inactivated by proteolytic enzymes, indicating them to be bacteriocin-like substances according to Jack, Tagg, and Ray (1995). According to Fricourt, Barefoot, Testin, and Hayasaka (1994), LAB synthesize bactericidal agents that vary in their spectra of activity. Many of these agents are bacteriocins with a proteinaceous active moiety, while others are non-protein agents (Piard & Desmazeaud, 1991, 1992). The high prevalence of bacteriocin-producing Lb. fermentum strains and their detection in different stages of doklu production indicate a high potential of these strains to grow and dominate the microbial population in the ecological environments of the food. The ability of Lb. fermentum species to inhibit a wide range of food spoilage fungi and bacteria is of special interest for food safety, especially in the Ivoryan environment with perennial problems of poor food hygiene. The use of such strains as a bio preservative could be a suitable means of enhancing the quality and safety of the local food products. Since the twenty bacteriocin producing Lb. fermentum showed many similarities (morphology, spectrum of inhibition, identical profiles of carbohydrate fermentation), they could be considered as being the same strain. Based on their inhibition activity, four strains were selected and examined in greater detail by PFGE. Results showed a great diversity between these bacteriocin producing Lb. fermentum species indicating the involvement of a variety of strains throughout the fermentation. Depouilly, Dufrene, Beuvier, and Berrther (2004) also showed such intrinsic diversity of LAB strains in the “Comité” cheese after PFGE analysis. Such a food matrix (fermented maize dough) therefore represents an abundant resource of potentially useful LAB for food bio preservation.

5. Conclusion

The present study has revealed the diversity and dynamics of LAB species involved in the traditional processing of doklu in Côte d’Ivoire. The initial stage of the fermentation was characterized by a codominance of Lb. fermentum, P. acidilactici, and P. pentosaceus, but eventually gave way to the dominance of Lb. fermentum and Lb. plantarum throughout the fermentation. Moreover, Lb. fermentum strains produce a bacteriocin and PFGE analysis associated to the dendrogram showed a high diversity between these LAB. To our knowledge, this is the first report using PCR-TTGE and 16S rRNA gene sequencing to describe the predominant bacterial diversity and dynamics in fermenting maize dough for doklu production. This approach has improved our knowledge of the bacterial populations present, their dominance and their dynamics in doklu processing. The peculiar antimicrobial characteristics of LAB strains can positively have impact on their use as starter cultures for traditional fermented foods, with a view to improving the hygiene and safety of the food products so produced.

Acknowledgments

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References


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