Journal of Advances in Biology & Biotechnology

Volume 26, Issue 3, Page 33-44, 2023; Article no.JABB.99556
ISSN: 2394-1081

Characterization and Functional Properties of Lactic Acid Bacteria Isolated from Fermented Maize Dough Used for the Preparation of *Doklu*

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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/JABB/2023/v26i3626

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/99556

Original Research Article

Received: 09/03/2023
Accepted: 13/05/2023
Published: 02/06/2023

ABSTRACT

Lactic acid bacteria (LAB) has long been safely applied in the production of fermented foods and beverages. These bacteria are important for their contribution to food quality and also for their undeniable effects on health. In Africa, despite the abundance of isolated food sources and the diversity of identified microorganisms, the market for probiotics and functional foods based on selected local strains is almost non-existent. This paper aims to determine some technological

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properties of LAB isolates from fermented maize dough used for *doklu* production for potential application in food and probiotic products. A total of 25 LAB strains isolated from maize dough fermentation processes during *doklu* production were identified by 16S rDNA gene sequencing. Technological studies such as growth and acidification kinetics, diacetyl production, hydrogen peroxide production, and proteolytic activity were carried out. Their survival and growth under unfavorable conditions of temperature, salt concentration and pH were also analyzed. The isolates were identified as *Pediococcus acidilactici* (56%), *Lactobacillus fermentum* (24%), *Pediococcus pentosaceus* (12%) and *Lactobacillus plantarum* (8%). The technological properties of the LAB strains showed that 10 of them rapidly acidify the medium with pH variation (ΔpH) greater than 1 after 6 hours of fermentation. Good production of diacetyl and hydrogen peroxide, as well as good proteolytic activity, were obtained for several strains. *Pediococcus acidilactici* S15, S55, S56; *Lactobacillus plantarum* S32, S121 and *Lactobacillus fermentum* S44 showed the best technological properties. However, only 5 of them obtained good resistance following the various survival and growth under unfavorable conditions of temperature, salt concentration, and pH. This work has therefore demonstrated that the lactic acid bacteria involved in the natural fermentation of maize dough have interesting properties for various applications whether in the processing of food products as well as in the development of probiotic products.

Keywords: Doklu; Lactic Acid Bacteria (LAB); maize dough; Pediococcus acidilactici; technological properties.

1. INTRODUCTION

Foods derived from cereals are popular worldwide and differ depending on the local culture and traditions [1]. They serve as important components of the daily diet, providing carbohydrates, proteins, dietary fibers, and vitamins [2]. *Doklu*, one of these cereal-based foods, is a product obtained from fermented corn dough consumed by people in southern and South-Easten of Côte d'Ivoire. People often appreciate *doklu* for its sour taste [3]. The manufacturing process of this product includes a fermentation step performed by a wide range of microorganisms, predominated by lactic acid bacteria (LAB). LAB involved in this spontaneous fermentation were mainly identified as *Lactobacillus fermentum, Pedioccocus acidilactici, Lactobacillus plantarum, Pediococcus pentosaceus,* and *Weissella cibaria* [4].

Studies on the technological and safety properties of LAB from different origins evidence that such microbial group has appropriate characteristics for many applications [5]. LAB has long been safely applied in the production of fermented foods and beverages. They occupy a central role in traditional starter cultures and are known to enhance the shelf life, and improve microbial safety, texture and sensory profile of the fermented product, mainly through the production of organic acids, aroma compounds, bacteriocins, exopolysaccharides and several enzymes [6]. LAB are also common components of probiotics, due to the fact that they are « generally regarded as safe » as they have long been used in the manufacture of foods and are desirable members of the intestinal microflora [7].

In Africa, despite the abundance of isolated food sources and the diversity of identified microorganisms, the market for probiotics and functional foods based on selected local strains is almost non-existent. Isolation of original LAB from traditional fermented foods is of crucial interest in view of there is paucity of literature regarding novel and emerging uses of LAB as probiotics in most developing countries. However, for the application of these LAB strains as dietary adjuncts, in addition to screening for beneficial properties, several factors which may influence the survival and colonization of these bacteria need to be addressed [8]. This paper aims to determine some technological properties of LAB isolates from fermented maize dough used for *doklu* production. We reported the isolation, characterization and identification of novel LAB strains. Also, the evaluation of some physiological and technological properties and the selection of some potential probiotic candidates.

2. MATERIALS AND METHODS

2.1 LAB Isolation and Identification

2.1.1 Sample Collection and isolation

Fermented maize dough samples were collected just at the end of the fermentation in sterile

containers from a traditional producer at Abidjan (Côte d'Ivoire). Immediately after collection, samples were transferred in an icebox to the laboratory within 1 h for analyses. Lactic Acid Bacteria isolation was performed after dilution of 10 g of maize dough diluted with 90 mL of sterile buffered peptone water (BPW). Successive decimal dilutions were then prepared and spread out on Man Rogosa and Sharpe (MRS) agar (Biokar, France). Plates were incubated under microaerobic conditions at 37°C for 48 h. Subsequently, colonies were randomly picked from the countable plate from each sample, purified by successive streaking on the same medium, and tested for Gram coloration and catalase production. Gram-positive and catalase-negative isolates were stored at -80°C in MRS medium containing glycerol (25%).

2.1.2 DNA extraction

Two ml of overnight bacterial culture were used for DNA extraction. Bacterial cells were pelleted by centrifugation for 10 min at 6000 rpm and protoplast by 1 h incubation at 37°C in 300 µL of 50 mM Tris·HCl (50 mM) EDTA (5mM) at pH 7.5 containing lysozyme (10 mg/mL), Mutanolysin (60 U/mL) and RNAse (200µg/mL). The total DNA was then extracted as previously described [9].

2.1.3 PCR Condition

The V1-V3 region of the 16S rRNA gene was amplified by using extracted DNA from isolated strains. PCR amplifications were performed in a final volume of 50 µL, containing 10% of PCR buffer; 2.5 mM of dNTP, 10 pM of each primer (V1-AGTCAGTCAGCCGAGTTTGATCMTGGCTCAG and V3-TATGGTAATTCAATTACCGCGGCTGCTGG), 5 U/µL of Taq polymerase and 500 ng/µL of DNA extract. Samples were amplified in a thermal cycler (GeneAmp PCR System 9700) programmed as follows: initial denaturation of DNA for 1 min at 94°C; 30 cycles (each of 1 min at 94°C, 1 min at 65°C and 1 min at 72°C) and a final extension 10 min at 72°C. PCR products were then separated by electrophoresis on a 1 % (w/v) agarose gel.

2.1.4 Sequencing

The amplified fragments were sequenced by Eurofins Genomics (Ebersberg, Germany) and taxonomic analysis was performed using Blast NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

2.2 Technological Characterization

2.2.1 LAB strain preparation

For analysis, the strains were subcultured in MRS broth and incubated at 37°C for 48 h under microaerobic conditions. Overnight (12h, 37°C) bacterial cultures were harvested by centrifugation at 4000 rpm for 10 min and washed with sterile BPW. The obtained pellets were resuspended in BPW at a final concentration of approximately 10^9 CFU mL^{-1} [10].

2.2.2 Preparation of maize extract (MFE) broth

Maize flour extract broth was prepared as follows: 500 g of maize grain was washed, steeped in 5 L of water for 12 h, and germinated at 25°C for three days. After the removal of the sprouts, the grains were milled into powder. The maize flour obtained was suspended in distilled water (300 g in 1 L) and boiled for 15 min. The obtained liquid phase was centrifuged at 4000 rpm for 10 min at 4°C. The obtained solution was sterilized by autoclaving at 121°C for 15 min and used as culture broth in subsequent experiments.

2.2.3 Diacetyl production

Diacetyl production was determined by HCl titration of fermented MFE [11]. A volume of 7.5 mL of hydroxylamine solution (1 M) was added to 25 mL of 24 h fermented MFE and to 25 mL of unfermented MFE broth (control). Both flasks were titrated with 0.1N HCl to a greenish-yellow end point using bromophenol blue as the indicator. The concentration of diacetyl produced was calculated as follows:

$$Ak = \frac{(R - S)(100E)}{W}$$

Where Ak (mg) is the quantity of diacetyl, R the volume (mL) of 0.1N HCl consumed in residual titration, S the volume (mL) of 0.1 N HCl consumed in the titration of sample, E the equivalence factor of HCl to diacetyl is 21.52 mg and W is the volume of sample (25 mL).

2.2.4 Hydrogen peroxide production

The hydrogen peroxide production in fermented MFE was also quantified bye titration. To 25 mL of this mixture, 25 ml of diluted H_2SO_4 (10%) was added. %). The preparation was then titrated with 0.1N potassium permanganate ($KMnO_4$). The

endpoint was the point at which the pale pink color persisted for 15s before decolorization. Each ml of 0.1N KmnO4 is equivalent to 1.701 mg of H_2O_2 [11]. The volume of H_2O_2 produced was then calculated as follows:

$$\mathrm{H_2O_2\ concentration\ de\ H_2O_2} = \frac{\mathrm{V(KMnO_4)} \times \mathrm{N(KMnO_4)} \times \mathrm{E} \times 100}{\mathrm{V(H_2SO_4)} \times \mathrm{V}}$$

$V(KMnO_4)$: volume of $KMnO_4$ (ml)
$N(KMnO_4)$: $KMnO_4$ normality
E : $KMnO_4$ equivalence factor
$V(H_2SO_4)$: volume of H_2SO_4
V : volume of sample

2.2.5 Proteolytic activity

The proteolytic activity of isolate was determined using skim milk agar [12]. Standard plate count agar supplemented with 10% sterile skim milk was inoculated by touch with a fresh bacteria culture previously grown on MRS agar. The inoculated plates were incubated at 37°C for 24h. Proteolytic activity was estimated by the halo diameter.

2.2.6 Temperature, pH and salt stress

Strains ability to grow in various stress conditions was evaluated using MRS broth inoculated with 1% of overnight culture (i) at an incubation temperature of 4, 30 or 45°C, (ii) with 2, 4, or 6.5 % NaCl (w/v) and (iii) at pH adjusted to 4 and 9.6 with HCl and NaOH respectively. The strains' thermoresistance was evaluated by their survival after for 30 min incubation at 62°C [13].

2.3 Statistical Analysis

Statistical analysis was carried out with XLSTAT™ software (version 2016.1). One-way analysis of variance (ANOVA) and Tukey's test were used to perform multiple comparisons between analyzed variables, with a significance level of $p < 0.05$.

Relationships among the technological characteristics of the isolates were determined by Principal Component Analysis (PCA) using R software (version 3.6.1). The discriminating variables were growth and acidification kinetics, diacetyl production, hydrogen peroxide production, and proteolytic activity.

3. RESULTS

3.1 LAB Species Identified

A total of 25 News isolates were selected and characterized by 16S (V1-V3 region) primers. As shown in Table 1, the sequencing of 16S rDNA and phylogenetic analysis revealed four species which are *Pediococcus acidilactici* (56%), *Lactobacillus fermentum* (24%), *Pediococcus pentosaceus* (12%) and *Lactobacillus plantarum* (8%).

3.2 Technological Properties

3.2.1 Growth kinetics

The results of the growth kinetics in MFE of the 25 LAB strains from Fermented maize dough are shown in Fig. 1A. Based on the measured optical density (OD) during fermentation, all strains displayed good abilities of growth in maize-based broth. Some strains such *Lactobacillus fermentum* S46 and *Pediococcus acidilactici* S141, contrarily to the others showed rapid growth in the earlier steps of fermentation, with an OD about 0,9 after only 6h. But at the end of fermentation, most of them displayed a good final OD value.

3.2.2 Acidification kinetics

The acidifying capacity of the 25 tested LAB strains was evaluated by determining their pH evolution during fermentation in MFE (Fig. 1B). All isolates were able to lower pH during fermentation. Ten (10) LAB strains (*Pediococcus acidilactici* S55, S16, S77, S79, *Lactobacillus fermentum* S46, S47, S44; *Lactobacillus plantarum* S121, S32, and *Pediococcus pentosaceus* S141) showed abilities to rapidly acidify the MFE broth, with ΔpH values greater than 1 after only 6h. The other isolates had a slow onset of acidification, but at the end of fermentation displayed high ΔpH values.

3.2.3 Diacetyl production

Diacetyl production in MFE broth was carried out by HCl titration. Most of the tested LAB strains produced more or less diacetyl. Seven out of 25 isolates (*Lactobacillus fermentum* S46, S47, S81, *Pediococcus acidilactici* S60, S56, *Lactobacillus plantarum* S121 and *Pediococcus pentosaceus* S141) show best production with more than 80 mg (Fig. 2A). For the other isolates, the production was average although some were very weak.

Table 1. Molecular identification of new LAB isolated from fermented maize dough

Strain ID	Closely related LAB Type strain	16S rRNA similarity (%)	Acession number
S1	*Pediococcus acidilactici*	98,98	OK427311
S5	*Pediococcus acidilactici*	98,78	OK427312
S8	*Pediococcus pentosaceus*	99,39	OK427313
S15	*Pediococcus acidilactici*	99,18	OK427314
S16	*Pediococcus acidilactici*	98,98	OK427315
S32	*Lactobacillus plantarum*	100	OK415420
S33	*Lactobacillus fermentum*	99,8	OK427317
S41	*Pediococcus acidilactici*	98,98	OK427318
S44	*Lactobacillus fermentum*	99,8	OK427319
S45	*Lactobacillus fermentum*	99,8	OK427320
S46	*Lactobacillus fermentum*	99,39	OK427321
S47	*Lactobacillus fermentum*	99,39	OK427322
S55	*Pediococcus acidilactici*	100	OK415422
S56	*Pediococcus acidilactici*	100	OK415423
S60	*Pediococcus acidilactici*	99,39	OK427325
S61	*Pediococcus acidilactici*	99,39	OK427326
S77	*Pediococcus acidilactici*	99,39	OK427327
S78	*Pediococcus acidilactici*	99,18	OK427328
S79	*Pediococcus acidilactici*	99,39	OK427329
S80	*Pediococcus pentosaceus*	99,39	OK427330
S81	*Lactobacillus fermentum*	99,8	OK427331
S121	*Lactobacillus plantarum*	100	OK415421
S130	*Pediococcus acidilactici*	98,98	OK427333
S141	*Pediococcus pentosaceus*	99,39	OK427334
S146	*Pediococcus acidilactici*	98,98	OK427335

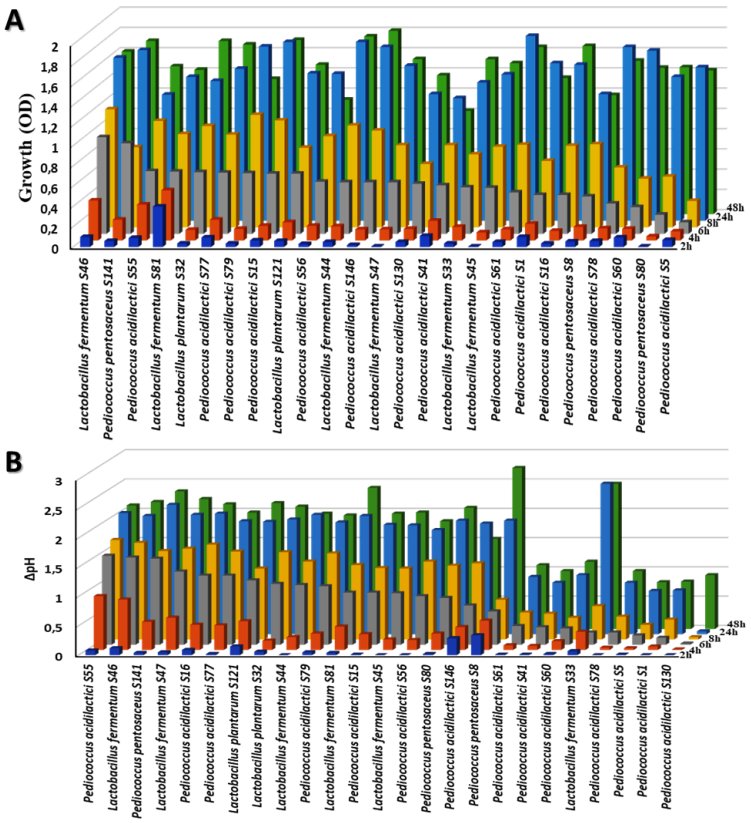

Fig. 1. Growth Kinetics (A) and acidification abilities (B) of LAB strains during fermentation in MFE broth

3.2.4 Hydrogen peroxide production

Potassium permanganate titration was used to determine hydrogen peroxide production by LAB strains. The concentrations of hydrogen peroxide produced are shown in Fig. 2B. Ten strains obtained a production greater than 2 mM with the highest quantity (2,99 mM) produced by *Pediococcus acidilactici* S15.

3.2.5 Proteolytic activity

Protease activity of LAB strains was assessed based on the ability to produce clear zone on skim milk agar (Fig. 3). Most strains achieved positive responses (Fig. 4). Five strains revealed a high level of hydrolysis of milk proteins with lysis diameters greater than 8 mm. Those are *Lactobacillus plantarum* S121, S32, *Lactobacillus fermentum* S44 and *Pediococcus acidilactici* S55, S56. All other strains had an average activity between 3 to 7 mm.

3.2.6 Salt, temperature and pH stress

The results of the tested LAB strains growth under different conditions are presented in Table 2. It was observed that all the strains grew at 30 and 45°C. However, at 4°C none of the tested strains was able to grow excepted *Lactobacillus fermentum* S45 and *Pediococcus pentosaceus* S80 which showed poor growth. At the concentration of 6.5% NaCl, *Lactobacillus fermentum* S45, *Pediococcus acidilactici* S56 and *Pediococcus pentosaceus* S80 displayed good growth, contrarily to the other strains. More or less growth was observed with most strains at NaCl concentrations of 2 and 4%. Moreover, all strains displayed growth at pH 4, however, this growth is low for some isolates. Except *Pediococcus acidilactici* S15 and *Lactobacillus fermentum* S45, reasonable growth was observed with all strains at pH 9.6. After 30 min of incubation at 62°C, all strains were able to grow, however, *Pediococcus acidilactici* S1 and S130 showed poor growth

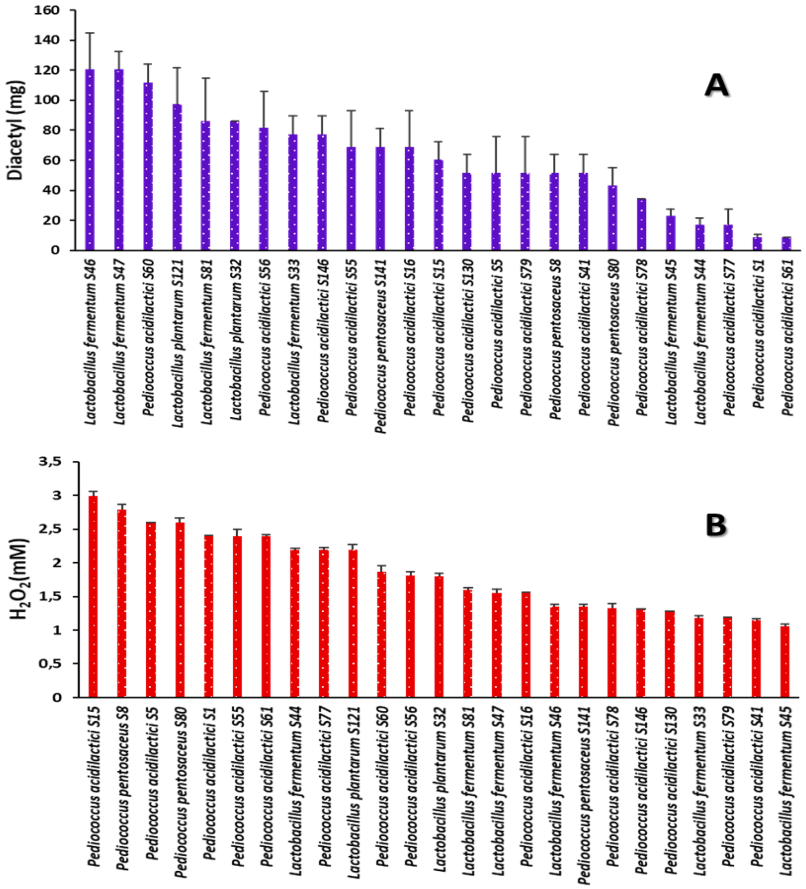

Fig. 2. Diacetyl (A) and hydrogen peroxide (B) production of LAB strains during fermentation in MFE broth

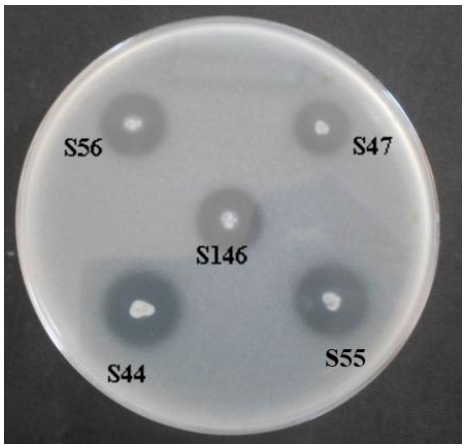

Fig. 3. Proteolytic activity of LAB strains on agar medium

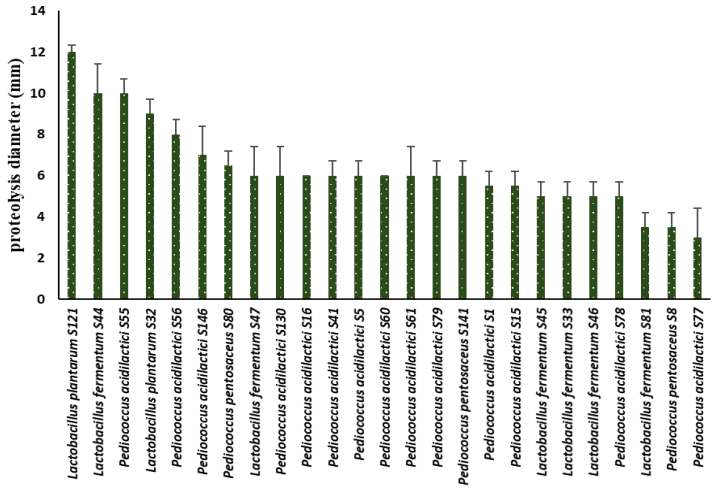

Fig. 4. Proteolytic activity of LAB strains

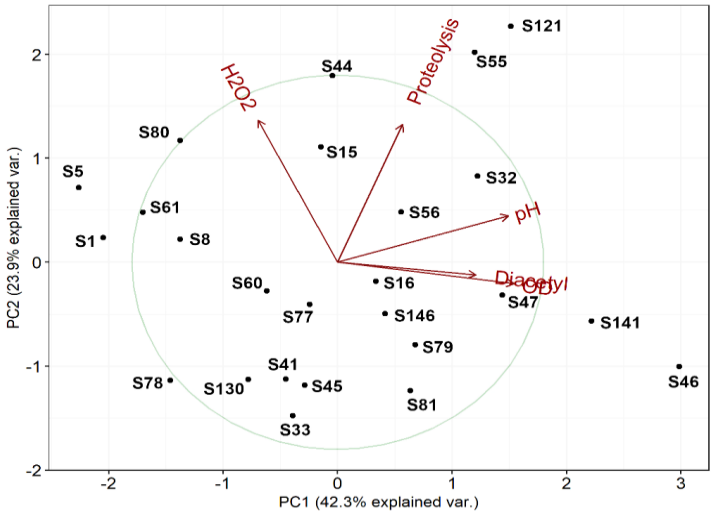

Fig. 5. Principal Component Analysis (PCA) of the technological characteristics of LAB isolates

Table 2. Growth characteristics of the strains at different pH, NaCl concentration, and temperature

Strains	Temperatures			pH		NaCl			Thermo-resistance
	4°C	30°C	45°C	4	9.6	2%	4%	6.5%	
Pediococcus acidilactici S1	-	+++	+++	++	++	++	++	+	+
Pediococcus acidilactici S5	-	+++	+++	++	+++	+++	+++	++	+++
Pediococcus pentosaceus S8	-	+++	++	+++	+++	+++	+++	++	+++
Pediococcus acidilactici S15	-	+++	++	++	+	+++	++	+	++
Pediococcus acidilactici S16	-	+++	+++	++	+++	+++	+++	+	+++
Lactobacillus plantarum S32	-	+++	+++	+++	+++	+++	+++	++	+++
Lactobacillus fermentum S33	-	+++	+++	++	++	++	++	-	+++
Pediococcus acidilactici S41	-	+++	+++	++	+++	+++	+++	++	+++
Lactobacillus fermentum S44	-	+++	+++	++	++	++	++	-	+++
Lactobacillus fermentum S45	+	+++	+++	+++	+++	+++	+++	+++	+++
Lactobacillus fermentum S46	-	+++	++	++	++	++	++	++	+++
Lactobacillus fermentum S47	-	+++	+++	+++	+	+++	++	+	+++
Pediococcus acidilactici S55	-	+++	+++	+++	+++	+++	+++	++	+++
Pediococcus acidilactici S56	-	+++	+++	+++	+++	+++	+++	+++	+++
Pediococcus acidilactici S60	-	+++	+++	+++	++	+++	+++	+	+++
Pediococcus acidilactici S61	-	+++	+++	+++	++	+++	++	-	+++
Pediococcus acidilactici S77	-	+++	+++	+++	+++	+++	+++	++	+++
Pediococcus acidilactici S78	-	+++	+++	++	++	+++	+++	++	+++
Pediococcus acidilactici S79	-	+++	++	+++	++	+++	++	+	++
Pediococcus pentosaceus S80	+	+++	+++	+++	++	+++	+++	+++	+++
Lactobacillus fermentum S81	-	+++	+++	+++	+++	+++	+++	+	+++
Lactobacillus plantarum S121	-	+++	+++	+++	+++	+++	+++	++	+++
Pediococcus acidilactici S130	-	+++	+++	+++	+++	+++	+++	++	+
Pediococcus pentosaceus S141	-	+++	++	++	+++	+++	+++	+	++
Pediococcus acidilactici S146	-	+++	+++	+++	+++	+++	++	+	+++

« +++ »: good growth; « ++ »: growth; « + »: poor growth; « - »: no growth

3.2.7 PCA analysis

The score plot (Fig. 5) shows the relationship among the technological parameters used in this study. Apart from Growth and Acidification kinetics, no strict relationship was found between the other parameters. Six strains form a distinct group in the positive direction for all variables. Those are *Pediococcus acidilactici* S15, S55, S56 ; *Lactobacillus plantarum* S32, S121 and *Lactobacillus fermentum* S44. They represent the bacteria with the highest activities for all variables combined.

4. DISCUSSION

LAB constitutes one of the most important bacterial groups used for the processing of fermented dairy, meat, vegetable, and cereal products [14]. In addition to their technological importance, many species of LAB act as probiotics [15]. When probiotics are added to foods, they must be able to survive in the product and become active when they enter the gastrointestinal tract of consumers. Therefore, in addition to assessing their probiotic potential, these cultures must also be tested for their functional and physiological properties [16]. In this study, 25 LAB strains isolated from fermented maize dough and identified by sequencing were subjected to different tests in order to select the most efficient ones for various biotechnological uses. Sequencing of the V1-V3 region of the 16S rRNA gene revealed the presence of four different species that are *Pediococcus acidilactici*,

Lactobacillus fermentum, Pediococcus pentosaceus and *Lactobacillus plantarum.* As already mentioned by other authors, these species of lactic acid bacteria are those commonly found in fermented maize dough [4,17]. These bacteria play an important role in the fermentation of grain-based foods. [18] related that, *Lactobacillus plantarum* and *Lactobacillus fermentum* are important species in fermented maize dough. The importance of *Lactobacillus fermentum* in maize fermentation has been confirmed by previous studies in Ghanan, Benin and Mexican products [19-22]. The high amylolitic activities found in different strains suggest that *Lactobacillus fermentum* could be a key organism for maize fermentation, making the large amounts of starch available to the overall community. In addition, the fermentation products (lactate, formate, and ethanol) may also serve as carbon sources for organisms, such as yeasts [17]. Interestingly, [4] reported a high prevalence of bacteriocin-producing *Lactobacillus fermentum* strains, and their detection in different stages of *Doklu* production indicates a high potential of these strains to grow and dominate the microbial population in the fermented maize dough.

Evaluation of acidification potential, proteolytic activity, diacetyl production and other tests have contributed to the technological and physiological characterization of the isolate LAB. Strains cultured for 48 hours in MFE were evaluated for their ability to grow correctly and to acidify the fermentation medium. The results obtained showed that all strains grow well in the broth. Some strains stood out for their rapid growth during the earlier stages of fermentation. After 24 h of fermentation, most strains reached an OD greater than 1 and this growth was independent of species. Knowledges on these growth parameters is necessary for more efficient industrial applications, considering the known nutritional requirement of this type of bacteria reported by some authors [23,24]. Acidifying activity is the most sought-after metabolic property of LAB used in the food industry. It is considered as an essential criterion for the selection of strains of technological interest. In this work, 75% of the strains showed a good acidification capacity with pH variation greater than 1.5 after 24 hours of fermentation. This good acidifying capacity of LAB has already been demonstrated by numerous studies [25-27]. The acidification capacity of these bacteria is due to the production of different types of organic acids that can contribute to make the environment unfavorable to the growth of certain microorganisms that could be undesirable from a hygienic point of view. Rapid acidification is also a priority in the development of lactic starter cultures. After only 6 hours of fermentation, 10 strains showed pH variation greater than 1, among which are represented the four identified species. Akabanda F et al. [28] had previously worked on the rapid acidification of LAB strains isolated from *Nunu* in Ghana, and these authors had also demonstrated the rapid acidification properties of *Lactobacillus plantarum* and *Lactobacillus fermentum* strains. The rapid acidification capacity of *Lactobacillus plantarum* has also been demonstrated by other authors [29,30].

The bacterial strains studied were also tested for the production of some metabolites of interest, namely diacetyl and hydrogen peroxide. Diacetyl is an essential aromatic compound. It is of industrial interest because it contributes to the flavor of many fermented foods. This aromatic compound is produced by lactic bacteria by citrate fermentation. Several bacteria of the genera *Lactococcus, Leuconostoc, Lactobacillus* and *Pediococcus*, can synthesize it [31]. In our study, the best production rate was obtained by seven strains including three *Lactobacillus fermentum*, two *Pediococcus acidilactici* and two *Lactobacillus plantarum*. In addition to contributing to the flavor of food products, diacetyl is characterized by broad antimicrobial activity [32]. It is able to inhibit Gram-negative bacteria and yeasts [33]. Hydrogen peroxide has also an antimicrobial effect. Its production was detected with all studied strains with concentrations higher than 2 mM for 10 of them. These results are in agreement with those obtained by [34] who tested 63 LAB strains and they were all good hydrogen peroxide producers. The hydrogen peroxide action can manifest itself both on undesirable germs and on those essential to the fermentation smooth running. Inhibition is achieved by oxidation of membrane lipids of target strains or by destruction of cellular protein structures [35].

Proteolytic activity of LAB strains was expressed on agar medium by lysis zones. According to [36], a strain is said to be correctly proteolytic if it presents a lysis zone diameter between 5 and 15 mm. In this study, 22 of the 25 strains tested (76%) obtained a proteolysis diameter greater than 5mm. LAB proteolytic systems play an important role in the ripening processes that give foods their rheological properties and

organoleptic characteristics [37]. The use of these bacteria as food additives may be an ideal factor to modulate the proteolytic activity of a food product [38]. It should be noted, however, that excessive proteolysis can lead to the uncontrolled production of bitter peptides and other undesirable compounds [39].

LAB survival and growth under unfavorable conditions of temperature, salt concentration and pH were determined. No remarkable growth was observed when cultivating the strains at 4°C. At 45°C, very good growths were recorded for all strains showing their ability to grow at temperatures above normal LAB growth temperatures. According to [40], most lactic strains grow in a temperature range between 15°C and 42°C. Strains that can remain viable at higher temperatures are called "thermoresistant". The 6.5% NaCl level allowed a good growth of only 3 strains out of the 25 studied strains. These are *Lactobacillus fermentum* S45, *Pediococcus acidilactici* S56 and *Pediococcus pentosaceus* S80. The ability of these strains to grow properly at 6.5% NaCl level could be distinctive since the literature generally presents an intolerance of lactic strains to this concentration [41]. Salt-resistant strains can be of technological interest especially in fermentations of salted products (salted cheeses, olives, cucumbers, meats, etc.) [42]. Regarding pH, the optimal values required for the growth and survival of bacteria are generally between 4 and 8. Under abnormal pH conditions, normal cellular components are not synthesized, which affects cell division and prevents growth [43]. Most of the strains studied were able to grow at pH 4, but at different intensities. Not surprisingly, pH 9.6 was the most critical. However, good growth was obtained for 13 of the strains. Despite treatment at 62°C for 30 min, 20 LAB strains showed good growth after incubation. It should be noted that during the drying of probiotics, the microorganisms are subjected to treatments involving extreme temperatures that can be very high (up to 200°C) during spray drying or very low (down to -196°C) during freeze drying and storage [44]. In general, resistance to these stresses is higher when the cells are previously exposed and adapted to a previous treatment with a homologous stress. For example, better heat tolerance was obtained when Lactobacillus rhamnosus GG was pre-exposed to 60°C for 20 min [45].

5. CONCLUSION

Based on partial 16S rRNA gene sequencing, 25 LAB isolated from fermented maize dough used for *doklu* production, were identified as *Pediococcus acidilactici*, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Pediococcus pentosaceus.* The obtained results show that certain isolates have promising technological properties for possible industrial applications. These strains have generally shown their capacity for good growth, acidification and the production of certain interesting metabolites such as diacetyl and hydrogen peroxide in a medium prepared from maize flour. They also demonstrated their ability to survive and growth under unfavorable conditions.

ACKNOWLEDGEMENTS

This research work was supported by funds from the International Foundation for Science (IFS) under the project number E/4955-2.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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