World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Selection of potential lactic acid bacteria starters with high lactic acid production capacity to control the fermentation of *néré* seeds (*Pakia Biglobosa*) in Côte d'Ivoire

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World Journal of Advanced Research and Reviews, 2025, 27(01), 2713-2720

Publication history: Received on 20 June 2025; revised on 28 July 2025; accepted on 30 July 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.1.2743

Abstract

The objective of this study was to select potential starters of lactic acid bacteria in order to control the fermentation of *néré* seeds (*Pakia Biglobosa*). During this study, some physicochemical parameters of *soumara* such as pH, titrable acidity, moisture content, and dry matter were initially determined. Fermented *néré* seeds were used to research lactic acid bacteria, which were then screened based on their fermentative technological characteristics. This study revealed that the pH of *soumara* (6.87) approaching neutrality led to an almost negligible value of titrable acidity (0.05 %). The dry matter content was 21.28 % with a high water content of 78.28 %. Thirty-three (33) lactic acid bacteria were isolated from *soumara* with 81.82 % cocci, 15.15 % bacilli, and 3.03 % coccobacilli. The study of the fermentation type revealed that 57.58 % of the isolates were homofermentative, while 42.42 % of the lactic acid bacteria were heterofermentative. Out of the 33 isolates, six strains showed a strong capacity for lactic acid production ranging between 1.67 % and 1.98 %, with good growth at high temperatures (30-50 °C) and the studied pH levels (5-8). Thus, the isolates BLS 08, BLS 09, BLS 13, BLS 20, BLS 28, and BLS 32 could be proposed as potential starters for controlling the fermentation of *néré* seeds.

Keywords: *Soumara;* Physico-Chemical; Lactic Acid Bacteria; Fermentative Type; Lactic Acid; Potential Starters

1. Introduction

Néré, an emblematic species of agroforestry widely present in the Sudanian savannas, is also known by its scientific name *Parkia biglobosa*. This food-bearing woody species is part of multi-use tree parks and belongs to the legume family, whose importance is recognized both regionally and internationally [1]. Considered as the quintessential field tree, it is of great utility to farmers thanks to the numerous functions it performs for the benefit of populations [2]. Indeed, traditionally transformed into fermented condiments under different names: *dawadawa* or *iru* in Nigeria [3], *nététu* in Senegal [4], *soumbala* in Mali and Burkina Faso [5], and *soumara* in Côte d'Ivoire [6], this product constitutes an invaluable source of goods and services for local communities in West Africa. *Soumara* is regularly used as a condiment in the preparation of various dishes and sauces, and plays an important role in the nutritional balance of populations as it often serves as a source of protein for low-income families [7]. *Soumara* is a popular condiment that enhances the flavor of various foods and has a cheese-like taste since it contains glutamic acid [8]. Ammonia, pyrazines, esters, acids,

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and ketones are among the chemicals that are formed during the fermentation of the seeds and are responsible for its potent smell and odor [9].

Three essential steps are involved in the manufacturing of s*oumara*: double cooking the grains, fermenting the cotyledons, and drying the fermented product [4]. The fermentation process leads to a notable improvement in the quality and nutritional value of *soumara* produced compared to that of the initial raw material [10]. This process fermentation occurs spontaneously with the development of the epiphytic microflora generally composed of *Bacillus spp*., particularly *Bacillus subtilis* [8, 11]. However, during fermentation, other microbiological species, such as lactic acid bacteria, are also present and are in charge of breaking down flatulent sugars in the cotyledons [11]. The use of lactic acid bacteria in food is interesting for more than one reason. Indeed, in addition to the fermentations for which they are responsible, lactic acid bacteria prevent the multiplication of other pathogenic bacterial species or those likely to degrade food products by producing organic acids and bacteriocins with proven antibacterial activity [12]. Thus, the identification of these microbial species responsible for the fermentation of *néré* seeds under environmental and processing conditions in Côte d'ivoire proves to be important. All this information will provide the scientific and technical data necessary for selecting appropriate starter cultures of lactic acid bacteria to control the production and quality of s*oumara*. It is in this context that the objective of this study is the selection of potential starter cultures of lactic acid bacteria in order to control the fermentation of *néré* seeds (*Parkia biglobosa*).

2. Materials and methods

2.1. Materials

The biological material is composed of fermented *néré* seeds. Following 72 hours of fermentation at a rate of 150 g per production unit, these seeds were collected under aseptic circumstances from three production units in the town of Korhogo (9° 27′ 41′′ North, 5° 38′ 19′′ West). For physico-chemical and microbiological analyses, these samples were transported in a refrigerated box to the Agropastoral Management Institute's Laboratory of Biochemistry, Microbiology, and Valorization of Agroresources at Peleforo Gon Coulibaly University of Korhogo.

2.2. Methods

2.2.1. Soumara seeds physico-chemical analyses

Measurement of dry matter and moisture in *soumara*

The moisture and dry matter content is that described by the AOAC method [13]. It is based on the dehydration of the samples by drying in an oven until a constant weight is obtained. Thus, five (5) grams of *soumara* samples were weighed in a glass capsule of known mass (m_0). The capsule containing the sample (total mass m_1) is placed in the oven (Memmert, France) set at 105 °C for 24 hours and is then placed in the desiccator to cool. The sample and capsule assembly is weighed (m_2) after cooling in the desiccator. The moisture content (H) expressed as a percentage of the mass of the wet sample is determined by the following relationship:

$$H(\%) = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100$$

The sample's dry matter (DM) content was calculated using the formula below and expressed as a percentage of its raw mass:

$$DM(\%) = 100 - H(\%)$$

Assessing the amount of ash in s*oumara*

AOAC method was used to determine the ash content (A) of *soumara* sample [13]. Five (5) grams (P_0) of *soumara* were mineralized in a muffle furnace (SX-4-10 Series, Tianjin, China) at 550 °C for six hours, or until all biological matter was completely destroyed. The ash was weighed (P_1) after cooling in a desiccator, and the ash content (%) was calculated using the formula below:

$$A(\%) = \frac{(P_0 - P_1) \times 100}{P_0}$$

Soumara's pH and titrable acidity

Soumara seeds pH was measured using the method outlined by N'goran-Aw et al. [14]. One hundred (100) mL of distilled water were mixed with 20 g of *soumara*. After filtration the resultant solution, the electrode of the previously calibrated pHmeter (Hanna, Germany) was submerged in it while being shaking. The pH meter's screen displayed the pH value directly. For determination of titrable acidity, 10 mL of the previously obtained filtrate were mixed with three drops of a 1 % phenolphthalein solution. A pale pink endpoint was reached by titrating the resultant solution against a 0.1 N NaOH solution [15].

2.2.2. Microbiological examination of soumara

In order to isolate and count the lactic acid bacteria, the stock solution was made by weighing 25 g of *soumara* and homogenizing them in 225 mL of buffered peptone water. Following that, decimal dilutions between 10^{-1} and 10^{-5} were carried out and MRS agar were inoculated with these decimal dilutions supplemented with 0.1 % of nystatin to prevent the growth of fungal strains. Lastly, spreading-inoculated agar plates were incubated at 30 °C for 48 hours. Petri plates with characteristics lactic acid bacteria colonies were chosen for counting following incubation. For enumeration, the standard formula was used to calculate the number of microorganisms, represented in CFU/g. Characteristic lactic acid bacteria colonies were identified using the following biochemical tests: Gram coloration and catalase test. Colonies identified such as lactic acid bacteria were stored in MRS broth supplemented with 20 % glycerol at -20 °C for any further tests [16].

2.2.3. Study of the technological properties of lactic acid bacteria and selection of potential starters

Investigation of acidification capacity and fermentative type of lactic acid bacteria

The acidification capacity and the type of fermentation of the bacterial strains were evaluated according to the methodology of Dicks and Van Vuuren [17]. In 10 mL of MRS agar supplemented with 2 % glucose and 0.005 % bromocresol purple (color indicator) and poured into a tube, each strain was inoculated by central puncture. After incubation at 30 °C for 72 hours, the change of the color indicator to yellow (acid production) with or without the presence of gas pockets allows for the determination of the heterofermentative or homofermentative fermentation type of the isolate, respectively.

Determination of acidifying capacity through lactic acid production

The isolates of lactic acid bacteria were pre-cultured in 5 mL of MRS broth. After incubation at 30 °C for 24 hours, 100 µL of this pre-culture was used to inoculate 10 mL of MRS broth. The incubation of the broths was carried out at 30 °C for 48 hours [18]. At the end of this incubation, samples were taken to determine cell growth, pH, and titrable acidity. Cell growth was determined by measuring the turbidity of the culture broths at 600 nm. The pH of the broths was measured using a pHmeter that has been previously calibrated. The titrable acidity, on the other hand, is determined by titration using a 0.1 N NaOH solution in the presence of 2 drops of phenolphthalein.

Investigation of the effects of temperature and pH on the growth of lactic acid bacteria isolates

The effect of temperature and pH on the growth of lactic acid bacteria isolates was carried out according to the protocol proposed by Yao et al. [19]. Five (5) mL of MRS broth were inoculated with 200 µL of pre-culture (OD600=1) of lactic acid bacteria. After 48 hours of incubation at different temperatures (30, 37, 40, 45, and 50 °C) and at different pH levels (5 to 8) at 30 °C, turbidity, which evaluates microbial growth, was measured by reading the optical density at 600 nm against a control.

2.3. Data analysis

All analyses were conducted in triplicate and the SPSS Statistics 20.0 software was used to determine the existence or not of a significant difference.

3. Results and discussion

The results of the physicochemical parameters of fermented *néré* seeds are recorded in Table 1. These seeds exhibit a pH value of 6.87 coupled with a very low titrable acidity of 0.05 %. Our pH and titrable acidity values obtained are similar to those reported in the literature by several authors who have obtained average pH values ranging between 6.6 and 7.45 [9, 20]. According to these same authors, the pH of unfermented seeds is around 5.24. The pH obtained in our study would be due to the activity of *Bacillus* bacteria during the fermentation of *néré* seeds. Indeed, these bacteria

during fermentation degrade the proteins present into simpler molecules such as amino acids while releasing ammonia, a compound that is responsible for the alkalinisation of the medium [8]. Fermented *néré* seeds have a high moisture content of 21.28 % correlated with a dry matter of 78.72 %. Research of Camara et al. [9] reported moisture contents ranging from 15.35 to 27.53 %, which are in line with our findings. Food goods' water content is a major factor in how they should be stored. The conservation and development time of microbial contaminants are greatly impacted by this characteristic [21].

Table 1 Physico-chemical properties of fermented *néré* seeds

Parameters	Unit	Values±SD
pH	-	6.87±0.03
Titrable acidity	(%)	0.05±0.0
Moisture	(%)	78.72±0.51
Dry matter	(%)	21.28±1.02

On MRS agar, they are small colonies with a whitish and creamy appearance, slightly raised, with a smooth outline. In total, 33 lactic acid bacteria were isolated from fermented *néré* seeds. These isolates have been numbered from BLS 01 to BLS 33. They are presented in the form of cocci (81.82 %), bacilli (15.15 %), and coccobacilli (3.03 %), Gram-positive and negative in the catalase test. Enumeration of lactic acid bacteria in the fermented *néré* seeds was 3.1-$\log_{10}$ cfu/g. Several research studies have reported the presence of lactic acid bacteria in fermentation in West Africa with cocci forms predominating [22, 23, 24]. However, their microbial load during fermentation is low due to the fermentation conditions (such as near-neutral fermentative pH). Thus, the lactic flora found considered subdominant compared to *Bacillus* bacteria, which are predominant. Although, LAB do not start the fermentation of *néré* seeds, but because they produce bacteriocin, their presence in the finished product improves its storage quality [25]. Gobbetti et al. [26] also noted that a number of sourdough lactic acid bacteria produce lactic acid, which delays the deterioration of bread and contributes to its aroma. *soumara* LABs might therefore have a similar function in preserving the result of *néré* seeds. Moreover, several research studies have mentioned the presence of certain species of lactic acid bacteria during the fermentation of *néré* seeds. These include *Lactococcus raffinolactus*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Weissella cibaria*, *Enterococcus faecium*, *Streptococcus oralis* and *Pediococcus sp*. [27, 23, 11].

Study of the fermentation type allowed for the grouping of isolated lactic acid bacteria into two groups. The first group contains homofermentative isolates with a percentage of 57.58 % (19 strains). In addition, the second group, with a proportion of 42.42 % (14 strains), includes the heterofermentative isolates. Moreover, all the isolates of lactic acid bacteria were able to acidify the MRS medium with a color indicator change. Our obtained results reveal a high proportion of homofermentative strains and are in agreement with the study by Coulin et al. [28], who isolated more cocci (57) than bacilles (43) in the traditional manioc ferment. In addition, research of Ouattara et al. [16] obtained a higher proportion of homofermentative isolates, with 90 % of lactic acid bacteria isolated from cocoa fermentation in Côte d'Ivoire. Indeed, these homofermentative isolates produce lactic acid only from carbohydrate fermentation via glycolysis, unlike heterofermentative isolates, which use the pentose phosphate pathway. They are capable of metabolizing sugars into lactic acid, ethanol or acetic acid and CO_2 [29].

The measurement of pH and titrable acidity in the present study allowed for the classification of lactic acid bacteria based on their acidifying capacity. The acidifying capacity varies depending on the isolates. Statistical analyses showed a significant difference between the pH of broths inoculated with the 33 isolates. The pH of the different culture broths ranged between 3.22 and 4.26 (Data no show) with a titrable acidity of 0.96 to 1.97 % after the incubation period (Table 2). Lactic acid production of the 33 lactic acid bacteria isolates allowed them to be grouped into three categories. Out of the 33 isolates, ten (10) lactic acid bacteria (30.30 %) were classified as weak lactic acid producers with values ranging from 0.96 to 1.26 %. Seventeen (17) isolates had lactic acid contents ranging between 1.27 and 1.66 % and were considered medium producers, accounting for 51.52 %. Finally, six (6) isolates were identified as strong producers, accounting for 18.18 %, with lactic acid values ranging between 1.67 and 1.98 %. BLS 09 strain exhibited the highest acidifying capacity (1.98 % lactic acid) with an MRS broth pH of 3.22, unlike the BLS 24 isolate whose culture broth showed a pH of 4.26 with a lactic acid quantity of 0.96 %. According to the findings, lactic acid bacteria isolated from *soumara* are distinguished by a high percentage of microorganisms with a high lactic acid production. These results are comparable to those of Sawadogo-Lingali et al. [30], who found that 43.80 % of the lactic acid bacteria isolates from sorghum paste caused the pH of the culture medium to drop. The main requirement for lactic acid bacteria, particularly

for strains meant for starter cultures, is acidification, which is defined by a drop in the pH of the culture medium. In fact, the bacteria pathogenic in *soumara* would be inhibited by the lactic acid that the lactic acid bacteria produce. Additionally, lactic acid preserves *soumara* better by acting as a biological preservative [31].

Table 2 Lactic acid production by lactic acid bacteria isolated from *soumara*

Fermentative capacity	Lactic acid (%)	Number of strains
Low producers	[0.96-1.26 %]	10
Middle producers	[1.27-1.66 %]	17
High producers	[1.67-1.98 %]	6

Figure 1 presents the influence of temperature on the growth of the six isolates of lactic acid bacteria with a high capacity for lactic acid production. The results obtained after reading the turbidity of the culture broths showed that temperature influences the growth of these lactic acid bacteria. Indeed, excellent growth of lactic acid bacteria is observed between 30 and 37 °C, followed by a significant decrease in growth when the temperature is increased from 40 to 50 °C. However, two trends emerge from this figure. The first trend concerns isolates BLS 20, 28, and 32, where an increase in cell growth is noted up to 37 °C. After this peak, a decline is reported. The second trend concerns isolates BLS 08, 09, and 13 with a decrease in cell growth up to 50 °C. This growth, however, is not null at 50 °C for all six isolates of lactic acid bacteria. According to Terpou et al. [32], the majority of lactic acid bacteria, the ideal growing temperature falls between 30 and 45 °C. Ouattara et al. [16], who demonstrated that lactic acid bacteria isolated from cocoa fermentation in Côte d'Ivoire are able to tolerate high growth temperatures, reported similar results. The ability of lactic acid bacteria to withstand high temperatures is a great advantage for the fermentation of *soumara*. Indeed, these isolates will be able to express all their potential as starters in controlled fermentations.

The ability of the six lactic acid bacteria to tolerate different pH levels is summarized in Table 3. All six isolates showed growth at the different pH levels studied, with a peak growth at pH 6 with optical densities ranging from 0.84 to 7.45. However, neutral and basic pH levels act by reducing cell growth compared to acidic pH levels. Research of Yang et al. [33] demonstrated that the optimal pH for lactic acid bacteria was between 6.2 and 8.5, and acidic pH inhibited the growth of these microbial strains. Furthermore, according to the research of Kambire et al. [31], the pH of *soumara* purchased on various markets in Côte d'Ivoire is between 6.1 and 6.8. Thus, the high cell concentration and lactic acid bacteria tolerance to various pH values suggest that lactic acid fermentations have a lot of promise for industrial use such as fermentation of *soumara*.

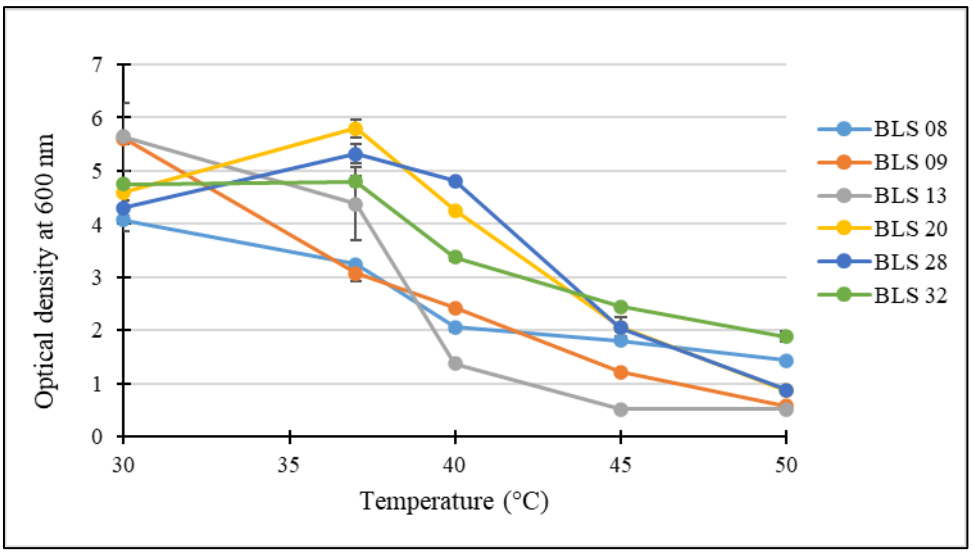

Figure 1 Influence of temperature on the growth of six isolates with high lactic acid production capacity

Table 3 Influence of pH on the growth of six isolates with high lactic acid production capacity

Isolates	Optical density at 600 nm			
	pH 5	pH 6	pH 7	pH 8
BLS 08	4.18±0.48^{a}	4.04±0.11^{a}	3.38±0.05^{b}	1.00±0.07^{c}
BLS 09	2.63±0.04^{b}	3.15± 0.05^{a}	2.38±0.04^{c}	0.43±0.06^{d}
BLS 13	0.19±0.01^{d}	0.84±0.01^{a}	0.65±0.03^{b}	0.46±0.01^{c}
BLS 20	3.47±0.19^{b}	3.97±0.06^{a}	2.08±0.07^{c}	1.03±0.00^{d}
BLS 28	4.81±0.07^{c}	7.45±0.10^{a}	5.85±0.11^{b}	2.49±0.08^{d}
BLS 32	2.37±0.01^{c}	5.27±0.02^{a}	4.51±0.03^{b}	0.91±0.02^{d}

Data are represented as means±SEM (n=3), Mean with different letters in the same line are statistically different ($p<0.05$) according to Duncan's test.

4. Conclusion

The present study was conducted with the aim of selection potential starter cultures of lactic acid bacteria for the control of the fermentation of *Pakia biglobosa* seeds. Nevertheless, some physicochemical parameters such as pH, titrable acidity, moisture content, and dry matter were measured. The results showed that the pH of the *soumara* is 6.87 with a titrable acidity of 0.05 %. The dry matter is 21.28 % with a moisture content of 78.72 %. Thirty-three lactic acid bacteria were isolated from *soumara* with 81.82 % cocci and 57.58 % homofermentative isolates. The production of lactic acid and the influence of pH and temperature on the growth of the isolates allowed the selection of isolates BLS 08, BLS 09, BLS 13, BLS 20, BLS 28, and BLS 32 as potential starters for controlling the fermentation of *néré* seeds.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared that there is no conflict of interest to be disclosed.

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