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Research Article

Isolation and Characterization of Yeasts and Lactic Acid Bacteria from Spontaneously Fermenting Pulp of Sorting Rejects of Kent and Brooks Mango Varieties

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Abstract

Kent and Brooks mango varieties are of major nutritional and economic importance to local populations. However, these fruits are highly susceptible to fermentation, leading to significant post-harvest losses. The objective of this study was to valorize sorting rejects of these mango varieties collected from two processing units in Korhogo (Côte d'Ivoire) through isolation of fermentative microorganisms from their pulps. After ripening, fermentative microorganisms (lactic acid bacteria and yeasts) were enumerated and characterized every three days over a nine-day period using specific culture media and standard microbiological techniques. Enumeration results revealed presence of lactic acid bacteria exclusively in Brooks variety, with microbial loads increasing up to day 6 (from 4.96 to 7.66 log CFU/g) before becoming undetectable from day 9 onwards. In contrast, yeasts were observed only in Kent variety, with a decrease in counts from day 0 to day 3 (from 4.97 to 3.57 log CFU/g), followed by complete disappearance by day 6. Furthermore, the characterization of the isolated fermentative microorganisms yielded a total of 45 lactic acid bacteria isolates and 30 yeast isolates from the pulps of Brooks and Kent mangoes, respectively. Characterization of lactic acid bacteria revealed 36 coccobacilli, 5 cocci, and 4 bacilli. Yeast isolates exhibited an oval morphology, with unipolar and multipolar budding observed as modes of reproduction. This study demonstrates that mango sorting rejects of Kent and Brooks varieties constitute a valuable source of fermentative microorganisms, including lactic acid bacteria and yeasts. The diversity and dynamics of these microorganisms during spontaneous fermentation highlight their potential for biotechnological applications, particularly in development of starter cultures. The isolation and characterization of these microorganisms provide a scientific basis for the valorization of mango sorting rejects, thereby contributing to the reduction of post-harvest.

Keywords

Mangoes, Sorting Rejects, Yeasts, Lactic Acid Bacteria

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1. Introduction

In Côte d'Ivoire, mango ranks as third most important commercial crop in northern region, after cotton and cashew, with an estimated annual production of 180,000 tons [1]. Despite this, local consumption and processing of mangoes, particularly Kent and Brooks varieties, remain very limited, leading to substantial post-harvest losses due to spoilage [2]. These varieties present challenges in storage and shelf life, largely because of the physiological changes that occur during ripening. However, high carbohydrate content of their pulps makes them an excellent substrate for studying fermentative microbial communities of biotechnological interest. Previous studies have indicated that the fruit microbiota is predominantly composed of bacteria, yeasts, and molds [3]. These microorganisms are widely exploited in biotechnology [4], owing to their ability to generate biomass through the synthesis or transformation of organic compounds [5]. Lactic acid bacteria are found in a wide range of food products, including fruits [6]. Their diverse metabolic capabilities explain their remarkable adaptability and ability to survive in numerous environments [7]. The role of lactic acid bacteria in food preservation is primarily associated with pH reduction through the production of organic acids [8]. Similarly, plant tissues such as fruits provide an ideal environment for yeasts. Alcoholic fermentation is carried out by yeasts due to the high sugar content and the abundance of nutrients in the fruit [9]. However, succession of microorganisms during fermentation can vary depending on local climatic conditions and physicochemical parameters of the fermentation medium [10]. Understanding fermentative microorganisms present in the pulps of Kent and Brooks mango sorting rejects is essential for their isolation and potential biotechnological applications. Therefore, this study was conducted to enumerate and characterize the fermentative microorganisms in pulps undergoing spontaneous fermentation of Kent and Brooks mango sorting rejects.

2. Materials and Methods

2.1. Material

Biological material consisted of pulps from sorting rejects of Kent and Brooks mango varieties (Figure 1), cultivated in the northern region of Côte d'Ivoire. The mangoes were collected from two processing units in Korhogo.

a) Kent pulp b) Brooks pulp

Figure 1. *Pulps from mango sorting rejects.*

2.2. Methods

2.2.1. Sampling and Preparation of Mango Pulps

Mature and unripe mangoes of the Kent and Brooks varieties, obtained from sorting rejects, were collected. Three (3) batches of twelve (12) mangoes per variety and per processing unit were sampled. For each variety, samples from the two processing units were combined to form a single batch. Mangoes were allowed to ripen at ambient laboratory temperature (25 °C) for 5 and 8 days for Brooks and Kent, respectively [11]. Pulps were then crushed and stored in Stomacher bags for subsequent analysis.

2.2.2. Isolation of Fermentative Microorganisms from Mango Sorting Reject Pulps

Stock suspension was prepared following method described by [12]. Briefly, 10 g of crushed pulp from Kent and Brooks mango varieties were mixed with 90 mL of sterile peptone water. The resulting stock suspensions were then used to perform decimal dilutions up to 10^{-7}.

Enumeration of lactic acid bacteria in spontaneously fermenting mango pulps was carried out on de Man, Rogosa, and Sharpe (MRS) agar according to AFNOR NF V08-052 standard. Inoculation involved spreading 0.1 mL of stock suspension and its decimal dilutions onto the surface of pre-poured and solidified MRS agar in Petri dishes, with three replicates

per dilution. Plates were incubated under anaerobic conditions at 37 °C for 72 hours. Typical lactic acid bacteria colonies were characterized by rough texture, serrated edges, smooth and opaque surfaces, either convex or spreading, with white, off-white, or yellowish coloration. Petri dishes containing 30-300 colonies were selected, and all colonies were counted.

NF ISO 6611 standard was applied for detection and enumeration of yeasts in spontaneously fermenting mango pulps. Oxytetracycline-Glucose-Yeast Extract Agar (OGA) was used for yeast identification and enumeration. Inoculation was performed by spreading 0.1 mL of stock suspension and its decimal dilutions onto surface of the pre-poured and cooled OGA in Petri dishes, with three replicates per dilution. Petri dishes were then incubated at 30 °C for 24 hours. Yeast colonies appeared as smooth, convex, and white. Petri dishes containing 15–150 colonies were selected for enumeration.

Germ counts were calculated according to ISO 7218 (1996), which determines number of colonies using the following formula:

$$N = \frac{\Sigma C}{(n1+0,1\times n2)_{d.v}}$$

2.2.3. Characterization of Lactic Acid Bacteria and Yeast Isolates

Cultural characteristics were used to identify differences among colonies observable to the naked eye [13]. For lactic acid bacteria isolates, characterization involved preparation of a smear followed by Gram staining. Microscopic examination was then performed under ×100 magnification using immersion oil, allowing determination of the Gram type and cellular morphology [14]. For yeast isolates, morphological analysis was carried out using methylene blue staining under a light microscope. A portion of each pure yeast isolate was mixed in a drop of sterile distilled water and placed on a slide for observation under ×40 magnification. Yeast cell shape and modes of reproduction were determined.

2.2.4. Statistical Analysis

Data obtained were processed using Microsoft Excel 2016. An analysis of variance (ANOVA) was performed using SPSS software to compare means at a significance level of $\alpha = 0.05$. Tukey's multiple comparison test was applied to assess differences between means. Results were expressed as mean ± standard deviation.

3. Results

3.1. Isolation of Fermentative Microorganisms

Figures 2 and 3 present the mean counts of lactic acid bacteria and yeasts, respectively, in pulps of Kent and Brooks mango varieties during fermentation. Enumeration results revealed presence of lactic acid bacteria exclusively in Brooks mango variety, while yeasts were detected only in Kent variety. Specifically, lactic acid bacteria count in Brooks variety increased from day 0 to day 6, rising from 4.96 log CFU/g to 7.66 log CFU/g, while no lactic acid bacteria were detected on day 9. Mean lactic acid bacteria counts in Brooks mango pulp were statistically different ($p < 0.05$). Regarding pulps of the Kent variety, a decrease in mean yeast counts was observed from 4.97 to 3.57 log CFU/g between day 0 and day 3, with a significant difference between two values. However, from day 3 to day 9, no yeasts were enumerated.

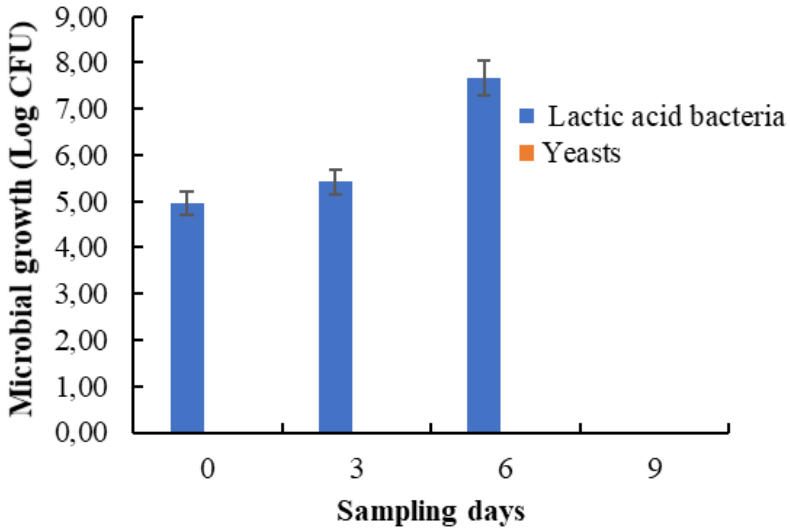


Figure 2. Lactic acid bacteria count in Brooks mango variety.

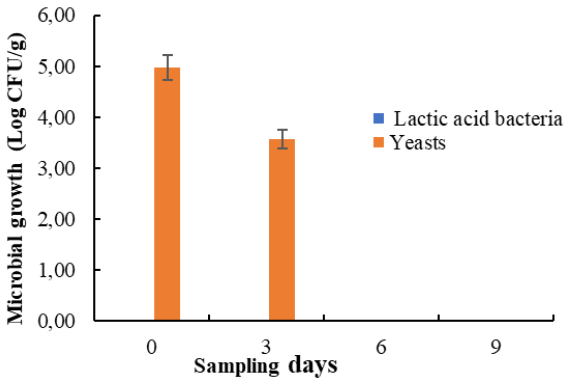


Figure 3. *Yeast counts in Kent mango variety.*

3.2. Characterization of Isolated Yeasts and Lactic Acid Bacteria

Figure 4 shows macroscopic appearance of isolated lactic acid bacteria and yeasts.

a) Lactic acid bacteria b) Yeasts

Figure 4. *Macroscopic appearance of isolated fermentative microorganisms.*

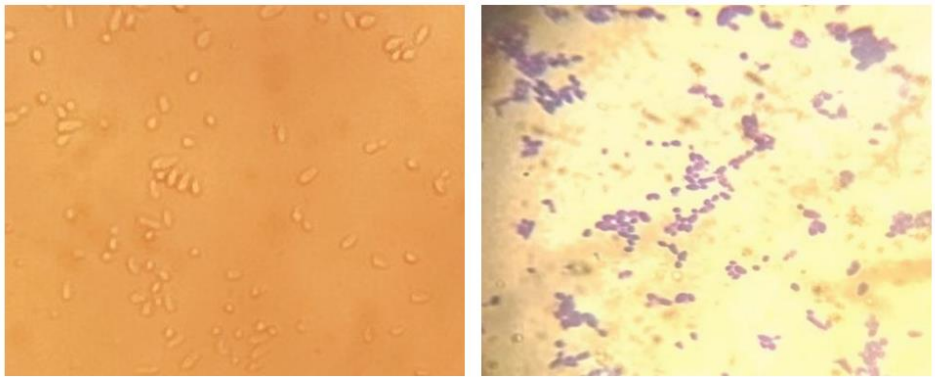
Isolation resulted in a total of 45 lactic acid bacteria isolates and 30 yeast isolates from pulps of Brooks and Kent mangoes respectively during fermentation.

Figure 5 presents results of microscopic examination of lactic acid bacteria isolates from Brooks mango pulps performed at ×100 magnification. All isolates exhibited a Gram-positive reaction which is characteristic of lactic acid bacteria. The isolates displayed bacilli, coccobacilli, and cocci morphologies. In total, 36 coccobacilli, 5 cocci, and 4 bacilli were observed from ripening stage (day 0) to fermentation stage (day 6) of Brooks variety.

a) Bacilli b) Coccobacilli c) Cocci

Figure 5. *Morphological characteristics of lactic acid bacteria isolates from Brooks mango.*

Figure 6 illustrates results of microscopic examination of yeast isolates from Kent mango pulps, observed in fresh state and after methylene blue staining at ×40 magnification. All isolates exhibited an oval shape and both unipolar and multipolar budding were observed as modes of reproduction. Yeasts reproducing by unipolar budding are likely to belong to genus *Pichia*, whereas those reproducing by multipolar budding are likely to belong to genus *Candida*.



a) Microscopic appearance of fresh yeasts b) Microscopic appearance of yeasts stained with methylene

Figure 6. *Morphological characteristics of yeast isolates from Kent mango pulp.*

4. Discussion

The study focused on isolation and characterization of indigenous fermentative microorganisms of interest present in pulp from sorting rejects of Kent and Brooks mango varieties. Pulp of Brooks mangoes was dominated by lactic acid bacteria, whereas Kent pulp was primarily colonized by yeasts. These microorganisms were selected for their biotechnological potential. In Brooks, lactic acid bacteria counts increased significantly from day 0 (4.96 log CFU/g) to day 6 (7.66 log CFU/g), followed by a decline on day 9. This pattern likely reflects an initial exponential growth phase succeeded by a stationary or decline phase, commonly observed in fermentable fruits and vegetables [15]. The early increase may be attributed to the high content of fermentable sugars in Brooks mangoes [16], while the subsequent decline could result from substrate depletion, accumulation of organic acids (predominantly lactic acid) and a reduction in pH, creating unfavorable conditions for bacterial growth [17, 18]. These observations are consistent with [19], who reported similar trends during cocoa fermentation. Absence of yeasts in Brooks pulp may be related to its specific biochemical composition.

Furthermore, pulp of Kent variety exhibited a decrease in yeast counts, from day 0 (4.97 log CFU/g) to day 3 (3.57 log CFU/g), followed by complete absence in day 6. High initial yeast load may be attributed to favorable growth conditions in Kent mangoes [20, 21]. The rapid decline during fermentation could result from a reduction in simple sugar content or presence of antimicrobial compounds such as polyphenols and organic acids, which limit yeast survival [22]. Indeed, certain fruits, including Kent mangoes are known for their high polyphenol content, which may confer natural antifungal activity [23]. Absence of lactic acid bacteria in this variety could be explained by its biochemical and physiological characteristics, which significantly influence presence and dynamics of fermentative microorganisms. According to [18], biochemical factors (pH, sugars, polyphenols) and physiological factors (maturity, texture, moisture content) are key determinants of bacterial or yeast presence and survival. Morphological analysis allowed characterization of lactic acid bacteria and yeast isolates from pulps of both varieties. Lactic acid bacteria isolates obtained on MRS medium were Gram-positive, a typical feature of this group [24]. Their morphology (coccobacilli, bacilli and cocci) corresponds to genera commonly found in fruits, such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Enterococcus* [25]. The high proportion of coccobacilli in Brooks pulp is comparable to findings reported by [26] in fermented fruits and vegetables, where *Lactiplantibacillus* coccobacilli dominate due to their adaptability to extreme, sugar-rich environments. Regarding yeast isolates, their oval shape and reproduction mode via unipolar or multipolar budding are characteristic of fermentative yeasts [27]. Distinction between unipolar and multipolar budding may guide classification toward the genera *Pichia* and *Candida*, respectively. These results align with [28], who reported that *Pichia kudriavzevii* and certain *Candida* species (*C. tropicalis* and *C. parapsilosis*) are responsible for spontaneous fermentation of tropical fruits.

5. Conclusion

This study aimed to identify the microorganisms present in spontaneously fermenting pulp of sorting rejects from Kent and Brooks mangoes. A high load of lactic acid bacteria was observed exclusively in Brooks pulp from day 0 to day 6, whereas yeasts were detected only in Kent pulp from day 0 to day 3. Microscopic analysis of yeast and lactic acid bacteria isolates indicated that these mangoes harbor a substantial diversity of fermentative microorganisms. The results suggest that the yeasts are closely related to genera *Pichia* and *Candida*, while lactic acid bacteria may belong to genera *Lactobacillus* and *Leuconostoc*.

Abbreviations

MRS	Man Rogosa, Sharpe
OGA	Oxytetracycline-Glucose-Yeast Extract Agar
ISO	International Organization for Standardization
AFNOR	French Association for Standardization
N	Number of Microorganisms (CFU/g)
ΣC	Sum of Colonies Counted on the Selected Petri Dishes at Two Consecutive Dilutions
n_1	Number of Petri Dishes Retained at the First Dilution
n_2	Number of Petri Dishes Retained at the Second Dilution
d	Dilution Corresponding to First Countable Plates
v	Volume of Inoculum Used
CFU/g	Colony Forming Unit per Gram

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Author Contributions

Safiatou Traore: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing

Tidiane Kamagate: Methodology, Supervision, Writing – review & editing

Abdoulaye Toure: Investigation, Methodology, Supervision, Validation, Writing – review & editing

Armel Fabrice Zoro: Methodology, Supervision, Writing – review & editing

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Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript

Conflicts of Interest

The authors declare no conflicts of interest.

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