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Biochemical and molecular characterization of yeasts and lactic acid bacteria isolated from *Borassus aethiopum* Mart. sap in Burkina Faso

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Abstract

In Burkina Faso, the Palmyra Palm *Borassus aethiopum* Mart. grows wild and gives natural stands in Central-Eastern and Eastern regions. The sap collected traditionally ferments spontaneously and is a rich medium that allows the growth of different microorganisms. This study aimed to identify yeasts and lactic acid bacteria (LAB) isolated from *Borassus aethiopum* Mart. fresh and fermented sap in Burkina Faso. A total of ninety strains including thirty LAB and sixty yeasts were isolated in the fresh and fermented sap. The isolates were characterized using standard biochemical method and sequencing of the V1 to V6 region of 16S rDNA of LAB and 28S rDNA of yeasts. The neighbour-joining method was used for the construction of phylogenetic tree with MEGA X software. After biochemical characterization and sequencing of the V1 to V6 region of 16S rDNA, twenty LAB strains (67%) were identified as *Leuconostoc mesenteroides*, seven (23%) as *Enterococcus sp.* and three (10%) as *Enterococcus gilvus*. Sequencing of the yeast 28S rDNA showed that 63% of the strains were *Saccharomyces cerevisiae* and 37% *Pichia kudriavzevii*. *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* are commonly isolated from several palm sap or wine of palm trees, but *Enterococcus* sp. and *Pichia kudriavzevii* are not commonly detected in palm wine. The LAB species *Enterococcus gilvus* identified in our study has not yet been isolated previously in palm wine. The yeasts and LAB isolated from *Borassus aethiopum* sap are the main microorganisms responsible for sap fermentation and could be used for several biotechnological applications.

1. Introduction

The palms largely widespread in the intertropical regions of Asia, America and Africa, offer many uses in the food, medicine, construction, craft, pharmacopoeia, fodder, energy, soil fertilization, agroforestry, etc. (Yaméogo *et al.,* 2008; Santiago-Urbina and Ruiz-Teran, 2014; Zongo *et al.,* 2018). Many common products and foods originate from palms trees but the most important product is palm wine which is an alcoholic beverage obtained by the natural fermentation of the sap from various species of palm (Hebbar *et al.,* 2018; Zongo *et al.,* 2019). In West Africa, it is consumed by more than 10 million people (Ukhum *et al.,* 2005) and in few quantities in Asia, South America, the Middle East and North Africa (Mbuagbaw and Noorduyn, 2012). This wine represents a source of income for rural peoples and an indispensable beverage in traditional African societies during various ceremonies. Palm wine is called by different names according to the country or region (Santiago-Urbina and Ruiz-Teran, 2014). In Africa, it is produced by natural fermentation of the sap of various palm trees species such as *Elaeis guineensis*, *Raphia vinifera, Raphia hookeri*, *Cocos nucifera*, *Hyphaene thebaica*, *Phoenix dactylifera*, *Borassus aethiopum*, *Borassus akeassii*, *Caryota urens* and *Nypa fruticans* (Mollet *et al.,* 2000; Amoa-Awua *et al.,* 2007; Ezeronye and Legras, 2009; Stringini *et al.,* 2009; Ziadi *et al.,* 2011; Ouoba *et al.,* 2012; Santiago-Urbina and Ruız-Teran, 2014; Tapsoba *et al.,* 2014; Karamoko *et al.,* 2016). The sweet sap of these palm trees is fermented spontaneously and transformed into a milky-white, flaky and effervescent beverage which shows the presence of

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fermentative microorganisms such as yeasts and bacteria (Okafor 1975; Ogbulie *et al.,* 2007). After the spontaneous fermentation of the crude sap, this wine becomes more and more acid and unacceptable for consumers after 3 days and develop a vinegary taste within a few days (Stringini *et al.,* 2009; Ouoba *et al.,* 2012). Most of the authors have reported that palm wine results from an alcoholic, lactic and acetic fermentation, it is a very nourishing drink that promotes lactation, improves eyesight, treats conjunctivitis and also serves as a sedative (Atputharajah *et al.,* 1986; Amoa-Awua *et al.,* 2007; Obahiagbon and Osagie, 2007; Mbuagbaw and Noorduyn, 2012; Ouoba *et al.,* 2012; Santiago-Urbina *et al.,* 2013; Santiago-Urbina and Ruíz-Terán, 2014). Palm wine involves three types of fermentation: lactic, alcoholic and acetic, making this traditional beverage an interesting environment where microorganisms with potential biotechnological applications can be isolated (Santiago-Urbina and Ruíz-Terán, 2014). Fermented beverages and foods have a unique place in most societies, because of their economical and cultural importance (Legras *et al.,* 2007). The alcoholic beverage plays a central role in traditional society, thus the microbiology and biochemistry of the fermentation process must be well understood.

In Burkina Faso, there are two types of Palmyra palm which are part of the natural stands widely used in the Western, Central-Eastern and Eastern regions as previously reported (Bayton *et al.,* 2006; Bayton and Ouédraogo, 2009). In these areas, the Palmyra extend over large areas of arable land. These are western palms consisting mainly of the *Borassus akeassii* species whose fruit are green at maturity and those of *Borassus aethiopum* in the East and Central-East with yellow fruit at maturity (Bayton *et al.,* 2006; Bayton and Ouédraogo, 2009). The western Palmyra *Borassus akeassii* are well valued through the exploitation of the sap for palm wine production called *bandji* which plays an important role for rural population (Yaméogo *et al.,* 2008; Ouoba *et al.,* 2012; Tapsoba *et al.,* 2014). The biochemical and microbiological characteristics of *bandji* are largely studied (Ouoba *et al.,* 2012; Tapsoba *et al.,* 2014; Tapsoba *et al.,* 2015). However, the eastern palm *Borassus aethiopum* Mart. is also important but is not highly valued. These stands are poorly valued; the sap is unknown and not exploited by local populations (Zongo *et al.,* 2018). Studies have been performed on various aspects of the socioeconomic importance of Palmyra *B. aethiopum* Mart. but few studies have been recently carried out on the physico-chemical and microbiological composition of its sap for beverage production (Zongo *et al.,* 2019). Yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) were the main fermentative microorganisms in *B. aethiopum* fresh and fermented sap (Zongo *et al.,* 2019). These microorganisms were isolated in this former study using standard microbiological methods but not identified. The identification of these organisms is, therefore, necessary because of their possible biotechnological potential. The aim of this study was to identify yeasts and LAB isolated from *B. aethiopum* sap in Burkina Faso using biochemical and molecular methods.

2. Materials and methods

2.1 Isolation of yeasts and lactic acid bacteria from Borassus aethiopum palm sap

Yeasts and lactic acid bacteria (LAB) were isolated from fresh and fermented sap from *B. aethiopum* in our former study according to the modified method described by Ouoba *et al.* (2012). Lactic acid bacteria were isolated on de Man, Rogosa and Sharpe Agar (MRS) (Sigma Aldrich, USA) containing Nystatin (100 mg/L) which were aseptically added to suppress yeast growth and plates incubated in anaerobic condition at 30°C for 72 hrs. Yeasts were isolated on Sabouraud Chloramphenicol agar (Biokar, France) incubated at 30° C for 72 hrs. After isolation, the strains were purified through several successive cultures on the same media. The pure strains obtained were stored at -20°C in Nutrient Broth supplemented with 30% glycerol for the yeasts. Strains of lactic acid bacteria were stored in Brain Heart Infusion broth supplemented with 30% glycerol at -20°C.

2.2 Biochemical characterization of yeasts and LAB strains

A total of ninety strains including sixty yeasts and thirty LAB were isolated from the fresh and fermented sap of *B. aethiopum*. Purified microorganisms were grown on appropriate media at 30°C for 48 hrs and then characterized. The biochemical characterization includes cell morphology, bacteria Gram stain, catalase and oxidase test, cell motility and sugars fermentation profile was carried out using standard methods in microbiology. The study of the fermentation profile of sugars of yeasts and lactic acid bacteria strains was carried out using a liquid base medium containing only the sugar to be tested as a carbon source. The liquid medium contained distilled water, 5 g/L of yeast extract, 1 g/L of KH_2PO_4, 1 g/L of NH_4CL, 0.5 g/L of $FeSO_4$ ($6H_2O$) and 3 g/L of sugar. The sugars that have been tested are glucose, fructose, sucrose, galactose, lactose, maltose, xylose, arabinose, ribose and mannose. Yeast strains and bacteria in the exponential growth phase (18-24 hrs) were used to inoculate the liquid medium containing Bromothymol Blue as a colored indicator. The pH of the

basal medium (green color) was adjusted to 7.2. The change in color from green to yellow after 24 to 48 hrs of incubation reflects the ability of the strain to ferment the sugar in question. The grouping of pure strains was based on their morphological, cultural, biochemical and physiological characters. Microorganisms with the same characteristics were placed in the same group. Some representatives from each group including four LAB and sixteen yeasts were selected and then sequenced to allow complete identification of the strains.

2.3 Sequencing of yeast and lactic acid bacteria strains isolated from Borassus aethiopum sap

The strains of yeasts and lactic acid bacteria were sequenced according to the SANGER method by Genoscreen society (Lille, France, www.genoscreen.com). The DNA was first extracted on a column from colonies of yeasts and bacteria previously grown on Sabouraud agar and MRS respectively using extraction kits. After extraction of the total DNA, the genomic targets were amplified by PCR with a HotStart polymerase (Thermo) to obtain two fragments of +/- 600 bp. The genomic target for lactic acid bacteria was the gene encoding 16S rDNA region V1 to V6. For yeast, the target is between 18S and 28S (LSU) of rDNA and integrates 5.8S. For yeasts, amplification was done on internal transcribed spacers (ITS) regions using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Finally, the PCR products were purified and a two-way sequencing was performed by the SANGER method on ABI 3730XL with the BigDye Terminator V3.1 kit. After validation, the sequences were cleaned then assembled and compared with the GenBank (National Center for Biotechnology Information, USA) database. The assembly of the forward and reverse sequences was done with Sequencher software version 4.7. The contigs (sequence in FASTA format) are then compared to the GenBank NCBI (http://blast.ncbi.nlm.nih.gov) database. The GenBank BLAST (Basic Local Alignment Search Tool) system makes it possible to find similarity regions between the sequences. The program compares nucleotide or protein sequences with sequence databases and calculates their statistical significance. In the NCBI, the database was determined on "Nucleotide collection (nr/nt)", and the program "Somewhat similar sequences (blastn)" was chosen to optimize the identification of yeast and bacteria strains.

2.4 Realization of phylogenetic trees

The Neighbor-Joining method was used to perform the evolutionary history or the phylogenetic tree of yeasts and LAB (Felsenstein, 1985; Saitou and Nei, 1987; Tamura *et al.,* 2004). The percentage of replication trees in the bootstrap test (1000 replicates) is indicated next to the branches. All ambiguous positions have been removed for each pair of sequences using the pairwise delete option. *Aspergillus niger* (NG065763) was used as an outgroup for yeasts and LAB. Evolutionary analyses were realized using the MEGA X software as described by Kumar *et al.* (2018). The algorithm MUSCLE was used for the multiple sequence comparison by log expectation as reported by Edgar (2004), multiple sequence alignments were constructed with the software.

2.5 Statistical analysis

The cleaning and the assembly of the forward and reverse sequences of strains were carried out via the software SEQUENCHER 4.7. (Genecode). The identification of the strains was done in GenBank NCBI (http://blast.ncbi.nlm.nih.gov) database by blasting the consensus sequences (BASTn). The MEGA X software was used for the realization of phylogenetic trees.

2.6 Sequence accession numbers in GenBank

The sequences of yeasts (16) and lactic acid bacteria (4) obtained in this study have been uploaded to GenBank NCBI (http://www.ncbi.nlm.nih.gov) with submission number SUB6432888, SUB6432908 and SUB6507317. The accession numbers were (BL30: MN577275.1; BL50: MN577276.1; BL58: MN577277.1; BL64: MN577278.1) for LAB and (Y125: MN585899.1; Y117: MN585900.1; Y130: MN585901.1; Y26: MN585902.1; Y38: MN585903.1; Y59: MN585904.1; Y98: MN585905.1; Y1: MN636439.1; Y4: MN636440.1; Y17: MN636441.1; Y21: MN636442.1; Y27: MN636443.1; Y31: MN636444.1; Y34: MN636445.1; Y39: MN636446.1; Y41: MN636447.1) for yeasts.

3. Results

3.1 Characteristics and isolates grouping

The presumed LAB (30) isolated from *B. aethiopum* sap were dominated by milky white colonies, circular shape, the size varied from 0.5 to 2 mm in diameter regular rods. All the isolates were Gram-positive, catalase-negative, oxidase negative and not motile (Table 1). The LAB isolates of group 1 were able to ferment saccharose, maltose, glucose, fructose, xylose, ribose and mannose but not lactose, galactose and arabinose (Table 2). The group 2 isolates fermented all the ten sugars tested excepted arabinose and group 3 ferment only saccharose, glucose and fructose. A total of 60 yeasts were isolated. The isolates were white, creamy, smooth, rough colonies, not motile, ovoid, spherical, opaque appearance and diameters between 1 and 5 mm. Based on the colony and cell morphology as well as the profile

Table 1. Biochemical characteristics of lactic acid bacteria isolated from *B. aethiopum* sap

Isolates	Groups	Cell morphology	Mobility	Gram	Catalase	Oxidase
BL59, BL60, BL49, BL58*, BL40, BL48,BL50*, BL41, BL52,BL55, BL37, BL46, BL47, BL74, BL73, BL28, BL32, BL76, BL42, BL23	1	Milky white colony, small, cocci, alone, in pairs or chain	–	+	–	–
BL27, BL30*, BL43	2	Milky white colony, small, cocci, alone or in chain	–	+	–	–
BL64*, BL69, BL70, BL53, BL65, BL26,BL44	3	Uncolourless colony, small, cocci, alone or in chain	–	+	–	–

Legend: (+): Positive reaction, (–): Negative reaction, + -: weak reaction ∗ : Isolate sequenced per group.

Table 2. Sugars fermentation profile of lactic acid bacteria isolated from *B. aethiopum* sap

Isolates	Groups	Saccharose	Maltose	Lactose	Glucose	Fructose	Galactose	Xylose	Ribose	Arabinose	Mannose
BL59, BL60, BL49, BL58*, BL40, BL48, BL50*, BL41, BL52, BL55, BL37, BL46, BL47, BL74, BL73, BL28, BL32, BL76, BL42, BL23	1	+	+	–	+	+	–	+/–	+/–	–	+/–
BL27, BL30*, BL43	2	+	+	+	+	+	+	+	+	–	+
BL64*, BL69, BL70, BL53, BL65, BL26, BL44	3	+	–	–	+	+	–	–	–	–	–

Legend: (+): Positive reaction, (–): Negative reaction, + -: weak reaction ∗ : Isolate sequenced per group.

Table 3. Sugars fermentation profile of yeasts isolated from *B. aethiopum* sap

Isolates	Groups	Catalase	Oxidase	Saccharose	Maltose	Lactose	Glucose	Fructose	Galactose	Xylose	Ribose	Arabinose	Mannose
Y21*, Y4*, Y17*, Y27*, Y1*, Y31*, Y39*, Y41*, Y34*, Y3, Y6, Y112, Y8, Y18, Y15, Y102, Y96, Y111, Y42, Y40, Y32, Y83	1	+	–	+/–	+/–	–	+	+	–	+/–	+/–	–	+
Y117*,Y26*, Y130*, Y59*, Y125*, Y98*, Y38*, Y10, Y19, Y28, Y9, Y20, Y69, Y23, Y33, Y5, Y126, Y116, Y95, Y121, Y127, Y129, Y30, Y29, Y24, Y97, Y120, Y114, Y66, Y48, Y67, Y36, Y7, Y25, Y14, Y115, Y2, Y36	2	+	–	+	–	–	+	+	+/–	–	–	–	+/–

Legend: (+): Positive reaction, (–): Negative reaction, + -: weak reaction ∗ : Isolate sequenced per group.

of fermentation of 10 carbohydrates, 2 different groups of yeasts were recognized. Nearly, all the isolates of yeasts were catalase positive, oxidase negative and mostly fermented saccharose, glucose, fructose and mannose, but not lactose and arabinose (Table 3).

3.2 Genetic identification

For identification of LAB and yeasts, the analyse of similarity was used to study the relationships between our isolates by comparing their 16S rDNA and 28S rDNA gene sequences with GenBank NCBI sequences

available by BLAST program. The following species could be affiliated to the alleged LAB: *Enterococcus gilvus* (BL30) with 100% similarity, *Leuconostoc mesenteroides* (BL50, BL58) with 100% similarity, and *Enterococcus* sp. (BL64) with 97.67% similarity (Table 4). The 28S rDNA gene sequence of yeasts isolates exhibited similarity to 99%−100% (NCBI) to the sequences available in these databases (Table 5). The selected yeasts clearly showed (Y125, Y117, Y130, Y26, Y38, Y59, Y98) 99%−100% similarity to *Saccharomyces cerevisiae*, (Y1, Y4, Y17, Y21, Y27, Y31, Y34, Y39, Y41) 100% similarity to *Pichia kudriavzevii* (Table 5).

3.3 Phylogenetic trees analysis

The phylogenetic trees determine the taxonomic affiliation of the species found in this study with the reference strains. The phylogenetic tree based on the 16S rDNA sequence (Figure 1) revealed that BL30 and BL64 strains were very close respectively to *Enterococcus gilvus* and *Enterococcus* sp. The strains of BL50 and BL58 were very close to *Leuconostoc mesenteroides* (Figure 1). Figure 2 illustrate the phylogenetic relationships derived from neighbour-joining analysis of 28S rDNA sequences of yeasts strains with the highest validated described species of the genus *Saccharomyces* and *Pichia*. The phylogenetic tree based on the 28S rDNA sequence further revealed that (Y125, Y117, Y130, Y26, Y38, Y59, Y98) and (Y1, Y4, Y17, Y21, Y27, Y31, Y34, Y39, Y41) were very close to *Saccharomyces cerevisiae,* and *Pichia kudriavzevii* respectively (Figure 2).

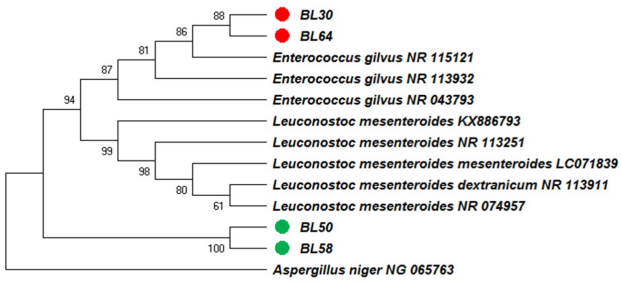

Figure 1. Phylogenetic tree of isolates and related lactic acid bacteria species based on 16S rDNA gene sequences. The tree was obtained by the neighbour-joining method, *Aspergillus niger* (NG065763) was used as an outgroup

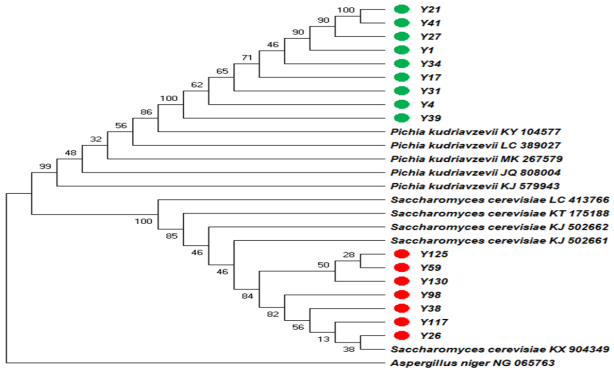

Figure 2. Phylogenetic tree of isolates and related yeasts species based on 28S rDNA gene sequences. The tree was obtained by the neighbour-joining method, *Aspergillus niger* (NG065763) was used as an outgroup

4. Discussion

The sap of the palm tree has been shown to be a rich medium that supports the growth of various types of microorganisms as reported by Amoa-Awua *et al.*

Table 4. Taxonomic identification of lactic acid bacteria isolated from *B. aethiopum* sap according to GenBank databases

Isolates	Identity	Similarity (%)	GenBank Accession Numbers
BL30*	*Enterococcus gilvus*	100	CP030932.1
BL50*	*Leuconostoc mesenteroides*	100	MK331874.1
BL58*	*Leuconostoc mesenteroides*	100	MK331874.1
BL64*	*Enterococcus* sp.	97.67	LC438519.1

Table 5. Taxonomic identification of yeasts isolated from *B. aethiopum* sap according to GenBank databases

Isolates	Identity	Similarity (%)	GenBank Accession number
Y125*	*Saccharomyces cerevisiae*	99.52	KJ502661.1
Y117*	*Saccharomyces cerevisiae*	99.88	KP674649.1
Y130*	*Saccharomyces cerevisiae*	99.88	KJ502661.1
Y26*	*Saccharomyces cerevisiae*	99.35	KJ502661.1
Y38*	*Saccharomyces cerevisiae*	100.00	CP006391.1
Y59*	*Saccharomyces cerevisiae*	99.98	KJ502661.1
Y98*	*Saccharomyces cerevisiae*	99.26	LC413766.1
Y1*	*Pichia kudriavzevii*	100.00	MK267579.1
Y4*	*Pichia kudriavzevii*	100.00	MK267579.1
Y17*	*Pichia kudriavzevii*	100.00	MK267579.1
Y21*	*Pichia kudriavzevii*	100.00	KF806465.1
Y27*	*Pichia kudriavzevii*	100.00	MK267579.1
Y31*	*Pichia kudriavzevii*	100.00	MK267579.1
Y34*	*Pichia kudriavzevii*	100.00	MK267579.1
Y39*	*Pichia kudriavzevii*	100.00	MK267579.1
Y41*	*Pichia kudriavzevii*	100.00	KF806465.1

(2007), Karamoko *et al.* (2012) and Zongo *et al.* (2019). The sweet sap of palm trees is fermented spontaneously and transformed into a milky-white, flaky and effervescent beverage due to the presence of fermentative bacteria and yeasts (Okafor 1975; Ogbulie *et al.,* 2007). *Saccharomyces cerevisiae* was the most yeasts species isolated in this study. This is in agreement with other studies carried out in Ghana by Brown (1994) who used restriction fragment length polymorphism to identify the yeast isolates and reported only the presence of *Saccharomyces cerevisiae* from the market samples of palm wine from several towns in southern Ghana. A similar observation was reported previously in Nigeria by Ezeronye and Okerentugba (2001). However, the study performed by Enwefa *et al.* (1992) reported the presence of several genera of yeasts including *Saccharomyces*, *Candida*, *Endomycopsis*, *Hansenula*, *Kleoclera*, *Pichia, Saccharomycoides* and *Schizosaccharomyces* in palm wine tapped from oil and raffia palms. Most of the authors have reported that *Saccharomyces cerevisiae* is the predominant yeast species in palm wine fermentations (Aidoo *et al.,* 2006; Amoa-Awua *et al.,* 2007; Stringini *et al.,* 2009; Tapsoba *et al.,* 2016). Bacteria and yeasts usually contaminate the juice as it is tapped and lead to the observed changes in biochemical composition of the palm sap (Atputharajah *et al.,* 1986). *Saccharomyces cerevisiae* is the predominant and best alcoholic fermenter among the yeasts responsible for palm wine fermentation (Stringini *et al.,* 2009). According to Opara *et al.* (2013), four micro-organisms in the succession order of Yeasts, *Micrococcus*, Lactic Acid bacteria and *Leuconostoc* spp. were found to be frequently present during the mixed culture fermentation of palm sap. The presence of potential endogenous microorganisms in the sap was emitted regarding the care applied in the collection of the crude sap (Ben Thabet *et al.,* 2010). *Leuconostoc mesenteroides, Enterococcus gilvus and Enterococcus* sp. were the LAB detected in *B. aethiopum* sap. Bacteria species such as *Corynebacterium sp., Lactobacillus casei*, *Lactobacillus paracasei* and *Leuconostoc sp.* were reported in *Borassus akeassii* palm wines (Tapsoba *et al.,* 2016). LAB constitute an important group of bacteria in beer and wine fermentations both as beneficial or spoilage organisms (Bokulich *et al.,* 2014). They are also responsible for its consistency owing to the production of gum, extracellular polysaccharide during the early stage of fermentation in the beverage. Amoa-Awua *et al.* (2007) and Ouoba *et al.* (2012) found *Lactobacillus plantarum* and *Leuconostoc mesenteroides* as the dominant LAB from palm wine in Ghana and Burkina Faso, however, some isolates of *Lactobacillus paracasei* were also reported. In fact, as reported by Rivera-Espinoza and Gallardo-Navarro (2010) and Mohamadou *et al.* (2010), species belonging to *Lactobacillus* and *Leuconostoc* genus are common bacteria reported in vegetable. The LAB is responsible for the acidification of palm wine, which not only gives its sour taste but also increases its stability. These species could enable the protection of palm wine against undesirable species. The study performed by Ouoba *et al.* (2012) reported in *Borassus akeassii* palm wine yeasts included mainly *Saccharomyces cerevisiae* and species of the genera *Arthroascus, Issatchenkia*, *Candida*, *Trichosporon*, *Hanseniaspora*, *Kodamaea*, *Schizosaccharomyces, Trigonopsis* and *Galactomyces*. The predominant yeast reported in *Saccharomyces cerevisiae*, but other yeasts such as *Schizosaccharomyces pombe*, *Kodamaea ohmeri*, *Hanseniaspora occidentalis*, *Candida tropicalis*, *Kloeckera apiculata* and *Pichia ohmeri* are also commonly detected in palm sap and wine (Atputharajah *et al.,* 1986; Aidoo *et al.,* 2006; Amoa-Awua *et al.,* 2007). The predominant genus of LAB from *Borassus akeassii* wine was *Lactobacillus* representing 86.67% of the total isolates followed by the genera *Leuconostoc* (10%) (Ouoba *et al.,* 2012). Stringini *et al.* (2009) investigated the occurrence of yeast flora during tapping and fermentation of palm wine from Cameroon using culture-dependent and culture-independent methods confirm the broad quantitative presence of yeast, lactic acid bacteria during the palm wine tapping process, and highlight a reduced diversity of yeast species with *Saccharomyces cerevisiae* as the predominant species similar reported in this study. *Saccharomyces cerevisiae* and *Candida tropicalis* have been reported in other palm wines such as those produced in Burkina Faso (*Bandji*), Nigeria (palm wine), and Sri Lanka (*Toddy*) (Atputharajah *et al.,* 1986; Ezeronye and Okerentugba, 2001; Ouoba *et al.,* 2012). Tra Bi *et al.* (2016) identified yeasts species in the raffia wine from Ivory Coast by sequencing the D1/D2 domain of the LSU rDNA and reported that *Saccharomyces cerevisiae* was the predominant, followed by *Kodamaea ohmeri*. The yeasts microflora is highly variable in palm sap or wine from different origins but *Saccharomyces cerevisiae* is the most common species isolated. *Pichia kudriavzevii* was also the predominant yeast in *Borassus aethiopum* fresh and fermented sap, but little identified in palm wine from various origins as mentioned by Tra Bi *et al.* (2016). The occurrence of non-*Saccharomyces* species and bacteria species varie according to several factors such as the region, palms species, composition of sap, the methods of palm wine tapping and collection of palm sap, etc. as reported previously (Tra Bi *et al.,* 2016). The dominated lactic acid bacteria were *Leuconostoc mesenteroides*. Lactic acid bacteria (LAB) are widely present in many fermented foods and beverages. Importantly, the presence of *Enterococcus gilvus* in *B. aethiopum* fresh

and fermented sap has not yet been isolated previously in other palm wine. The differences in environmental conditions, palm trees species, and the tapping process are likely the causes of the observed variations in the yeasts and bacteria species from palm sap or wines of different countries.

5. Conclusion

The results of this study revealed that the sap from *B. aethiopum* contained a low diversity of yeasts and LAB. Two genera of yeasts *Saccharomyces* and *Pichia* were found in fresh and fermented sap, the predominant species was *Saccharomyces cerevisiae*. The LAB genus found were *Enterococcus* and *Leuconostoc* with a predominance of the specie *Leuconostoc mesenteroides*. After biochemical characterization and sequencing, twenty LAB strains (67%) were identified as *Leuconostoc mesenteroides*, seven (23%) as *Enterococcus* sp. and three (10%) as *Enterococcus gilvus*. Sequencing of the yeast showed that 63% of the strains were *Saccharomyces cerevisiae* and 37% *Pichia kudriavzevii*. The yeasts and LAB identified in *B. aethiopum* sap represented potential uses for beverages production.

Conflict of interest

There is no conflict of interests for this study and all the authors agreed to the publication of the work.

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