Scientific Research Publishing

Advances in Microbiology, 2019, 9, 646-655
http://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Isolation and Identification of Indigenous Yeasts from "*Rabilé*", a Starter Culture Used for Production of Traditional Beer "*dolo*", a Condiment in Burkina Faso

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How to cite this paper: Mogmenga, I., Dadiré, Y., Somda, M.K., Keita, I., Ezeogu, L.I., Ugwuanyi, J., and Traoré, A.S. (2019) Isolation and Identification of Indigenous Yeasts from "*Rabilé*", a Starter Culture Used for Production of Traditional Beer "*dolo*", a Condiment in Burkina Faso. *Advances in Microbiology*, **9**, 646-655.
https://doi.org/10.4236/aim.2019.97040

Received: May 27, 2019
Accepted: July 28, 2019
Published: July 31, 2019

Abstract

Rabilé is a ferment of *dolo*, a traditional sorghum beer of Burkina Faso and it is harvested at the end of *dolo* fermentation process. It is a significant source of proteins and it is used like a condiment and food seasoning by some communities. The present study characterized and identified the yeasts isolated from *Rabilé*. A total of 70 samples were collected from 7 localities of Burkina Faso with *dolo* sellers according to the ethnic groups. The aerobic mesophilic flora count from *Rabilé* varied from 8.34 ± 0.72 to 10.07 ± 0.51 $\log_{10}$ CFU·g^{-1} and yeast varied from 7.24 to 8.28 $\log_{10}$ UFC·g^{-1}. Based on morphological, cultural, sexual and biochemical (carbon and nitrogen assimilation) profiles, 50 yeast strains were identified and found to belong to 4 genera and 7 species. *Saccharomyces cerevisiae* was found as most predominant yeasts species of yeast in of Rabilé with 64 %, *Rhodotorula muciloginosa,* (8%), *Candida pseudorhangii* (6%), *Candida heliconiae* (12%), *Candida utilis* (4%), *Shizosaccharomyces pombe* (4%) and *Sporobolomyces odoratus* (2%). This activity has been carried out in the Laboratory of Biotechnology for Food and Nutritional Sciences, University Ouaga I Pr Josph KY-ZERBO, Burkina Faso, and it was done from June to October 2018.

Keywords

Rabilé, *dolo*, Yeasts, *Saccharomyces cerevisiae*, Burkina Faso

1. Introduction

Dolo is a popular traditional beer of Burkina Faso. It is prepared from Sorghum bicolor and in certain localities, some others cereals such as millet and maize can be used as adjunct or substitutes [1] [2]. Traditional Sorghum bicolor beer is also the most popular alcoholic beverage in West Africa. They are known as *pito* in Ghana, *burukutu* or *otika* in Nigeria, *tchoukoutou* and *chakpolo* in Benin and Togo, *dolo* in Mali and *tchapalo* in Ivory Coast [3]. They are largely consumed during traditional ceremonies and constitute also an important source of income for the sellers and producers who are mostly rural men and women. The studies carried out by several authors on Africa traditional beverage showed that lactic acid bacteria and yeast strain *Saccharomyces cereviciea* are the most important microorganisms in these beverage [3] [4]. Other studies carried out have shown the probiotic properties of those microorganisms [5] [6] [7].

Studies carried out on Single Cell Protein production showed that yeast strains can be isolated from Africa traditional beverage and used as inoculum for food and feed protein production. Indeed, yeasts are able to assimilate several types of raw material and contain more than 50% total proteins with high content of essential amino acids. Also, it is an important source of vitamins (group B) and mineral salts [8]. Wijeyaratne and Jayathilakf [9] showed that *Candida* sp. contains vitamin B (riboflavin (0.231 mg/g), and thiamin (0.178 mg/g). Ouedraogo [10] revealed that *Candida utilis* NOY1 contains 54.8% ± 0.12% of protein. Somda [11] used *Saccahromyces cerevisae* produced yeast biomass with an interesting rate of essential amino acids using mangoes residues as substrate.

In Burkina Faso, lactic acid bacteria and yeasts are reported as the main microorganisms of dolo [3] [5]. The ferment of dolo, called Rabilé in Burkina Faso, Kpètè-Kpètè or Otchè in Benin, Umusemburo in Rwanda [2] [3] [6] [12] is used as condiment for some communities in Burkina Faso as protein source. It is generally harvested from the bottom of a previous fermenting beer resulting from 12 to 13 h overnight fermentation. Mainly studies have been done on *dolo* production process. However, the microorganisms contained in the *Rabilé* that is used as a protein source in food preparation has not yet been investigated. The increasing interest for yeast application like protein source, and thus ensuring food quality and safety, the identification and classification of the stains involved is very necessary.

The aim of this study is to determine the microbiological characteristics of the traditional beer starter "Rabilé" which is used as condiment, and to identify the different species of yeasts involved using phenotypic analysis tools. This is important to ensure that quality and purity of starter and to avoid contamination from pathogenic microorganism which may imperil the process and safety of product.

2. Material and Methods

2.1. Sample Collection

Before collecting the sample, some information about the production of Rabilé

has been collected with the sellers and the producers. An article on the quality of Rabilé used as condiment in Burkina Faso has been published in African Journal of Biotechnology.

The ferment of local beer "*Rabilé*" was collected from 70 (seventy) sellers of local beer in 7 (seven) localities Ouahigouya, Fada N'Gourma, Réo, Bobo Dioulasso, Grarango, Tougan Dissin (**Figure 1**) representing different ethnic regions of Burkina Faso. From each producer, one hundred grams (100 g) of "*Rabilé*" was sampled and maintained at 4˚C in isothermal box for laboratory analysis.

Enumeration of total aerobic mesophilic flora and yeasts

Each sample of *Rabilé* was properly mixed to ensure homogenization of the microorganisms present in the fermented product. Triplicate samples of *Rabilé* (10 g) were introduced aseptically into 90 ml (1:10 dilution) of buffered Peptone water (0.85% (wv-1) Biomerieux, France) into stomacher blister and stored at ambient temperature for 30 min. The mixture introduced in a McCartney bottle was mixed with a vortex (Gemmy Industrial Corporation, Italy) for 5 min. Decimal dilutions were plated and 0.1 ml of each of dilution was pipetted in duplicate into appropriately marked Petri dishes. Total count of total aerobic mesophilic flora was determined on plate count agar (PCA) after 24 h incubation at 30˚C and yeasts was determined on Sabouraud Dextrose Agar containing chloramphenicol (50 $\mu \cdot ml^{-1}$) after 3 to 5 days of incubation at 30˚C, under aerobic condition. Fifty (50) yeast strains were isolated and subjected to morphological, cultural, biochemical, fermentation and assimilation tests.

2.2. Identification of Yeast

Isolated colonies with distinct morphological appearance were picked up aseptically and subjected to several tests for identification. The classical methods used by [13] based on examination of morphological, biochemical, cultural and physiological properties of yeasts isolated in pure cultures were applied using Wickerham medium. Preliminary confirmation was based on microscopic observation. The isolates were tested for the fermentation of sucrose, lactose, glucose

Figure 1. Map of Burkina Faso. Colored parts = sites of sample.

and raffinose, as well as the assimilation of selected nitrogen sources: nitrate, ethylamine, L-lysine, cadaverine and sulfate of ammonium. The assimilation of carbon sources was performed using API 20 C AUX strips (BioMérieux, Lyon, France) according to the manufacturer's instructions. The diazonium blue B reaction, a test to differentiate between ascomycetous and basidiomycetous yeasts, was performed as described by [14].

2.3. Data Analysis

For the analytical data, mean values, as well as standard deviation, are reported. The data were analyzed using the statistical program, XLSAT 2014.5.03. The on-line available software (http://http:www.cbs.knaw.nl) of Centraal bureauvoor Schimmel cultures (Central Bureau of Fungal Cultures), Utrecht, the Netherlands was used for identification of yeasts.

3. Results and Discussion

3.1. Microbial Enumeration in *Rabilé*

Table 1 shows an enumeration of flora aerobic mesophilic total (FAMT) and yeasts found in *Rabilé* collected from different sampling sites. The results were converted to $\log_{10}$ CFU·g^{-1}. The average values of FAMT ranged between 8.42 ± 0.34 and 10.07 ± 0.18, while the amount of yeasts ranged between 7.24 ± 0.26 and 8.28 ± 0.20.

The results of FAMT and yeasts enumeration from *Rabilé* (Table 1) shows that FAMT and yeasts counted in *Rabilé* were higher than FAMT and yeast counted in a sorghum beer *burukutu* (7.95 ± 0.45 and 7.49 ± 0.54) as reported by [15]. Djêgui [6] thought that the difference between yeasts counted in *kpete-kpete*, *tchoukoutou* ferment and in *burukutu* could be due to the fact that there is a significant difference between the dry matters of both products. The results of Table 1 show there is a significant difference between FAMT and yeasts counted from the localities. However, the number of FAMT and yeasts

Table 1. Microbiological characteristics of the traditional starter Rabilé.

Localities	FAMT (log cfu/g)	Yeasts (log cfu/g)
Fada N'Gourma	9.2 ± 0.12^{b}	8.22 ± 0.31^{a}
Ouahigouya	9.37 ± 0.08^{b}	7.81 ± 0.16^{ab}
Tougan	10.07 ± 0.16^{a}	8.27 ± 0.20^{a}
Garango	8.41 ± 0.34^{c}	7.23 ± 0.26^{c}
Bobo Dioulasso	9.31 ± 0.21^{b}	7.8 ± 0.14^{abc}
Réo	8.86 ± 0.30^{bc}	7.75 ± 0.23^{abc}
Dissine	8.33 ± 0.27^{c}	7.26 ± 0.13^{bc}
Means	9.07	7.76

*Values with the same letter in the same column are not significantly different ($P < 0.05$), FAMT: Flora Aerobic Mesophilic Total, cfu: Colony Forming Unity.

counted from *Rabilé* is similar to the other authors found in alcoholic beverages fermented. Indeed, Djegui [1] and Keita [3] found 9.24 and 10.35 $\log_{10}$ CFU·g^{-1} of yeasts respectively in *Kpete-Kpete* of Benin and *Rabilé* of Burkina Faso. Also, Atter [15] counted 8.41 ± 0.38 $\log_{10}$ (UFC·g^{-1}) after the wort fermentation. The higher rate of FAMT could be due by contamination by other microorganisms. Mogmenga [2] showed that *Rabilé* used as condiment by certain communities of Burkina Faso is contaminated by *Staphylococcus aureus* and total coliform. The utilization of *Rabilé* as condiment could be a food contamination source.

3.2. Phenotypic Characteristics of Yeasts Isolates

Table 2 presents the morphological, cultural and biochemical characteristics of yeasts strains isolated from *Rabilé*. According to these characteristics, yeasts strains have been gathered into three groups. The group that presented a main difference with other groups was a group III. The color of those strains was red, and in broth medium there is not sail formation. The difference between members of group I and group II was the number of ascospores, there were 8 ascospores in the strains of group I and 4 ascospores in the strains of group II. All of strains of these groups were catalase$^+$ and oxidase$^+$.

3.3. Assimilation Profile and Identification of Yeasts Isolates

Considering carbon (API 20C AUX gallery) source assimilation profile (Table 3), fourteen (14) different groups of yeasts were distinguished. The majority of yeasts strains assimilated glucose (100%), galactose (74%), saccharose (66%) and maltose (100%). Others assimilated L-arabinose, D-Xylose, D-sorbitol, Methyl-α D-Glucopyranoside, D-raffinose and D-trehalose. However, none of them assimilated Calcium 2-keto-Gluconate, Inositol, N-acetyl-glucosamine and D-cellobiose.

Table 2. Morphological, cultural and biochemical characteristics of yeasts strains isolated form *Rabilé*.

Characteristics	Microorganisms		
	Group I	Group II	Group III
Cell morphology	Ovoid and spherical	Ovoid and spherical	Ovoid and spherical
Budding/Pseudo-mycelium	+/+	+/+	+/−
Aero-anaerobic facultative	+	+	−
Spores	+	+	+
Colonies color	White	White	Red
White sail formation/Sediment	+/+	+/+	−/+
Turbidity	+	±	−
Oxidase/Catalase	+/+	+/+	+/+

Group I: Oy3-3; Kb3-4; Oy3-10; Bf1-1; Tk3-8; Tk3-8; Ga3.10; Kb2.9, Og3.2, Og2.3, Dr1.6, Ga2.1; Fd2.10, Bf2.7, Bf2.2, BB1.7, BB1-1, Dr1-6, Tk3-10, Oy1-7, Fd3-9, Bf3.4, Bf2.6, Oy3.3, Oy3.10, Tk3.7, Kb3.4, Bf1.1, Bf2.5, Oy2.5, Bf1.8, Bf2.3, Ga3.7, Ga2-3, Fd3-3, Tk2-1; Group II: Fd2-2, Oy3-8, Oy1-5, Tk2-6, Oy2-9; Ga2-5; Dr1-9; Ga3-3; BB1-6; Tk3-9; Group III: Tk2-9, BB1-9, BB3-3, BB3-1; (+) Presence and positive reaction; (−) Absence and negative formation.

Table 3. Assimilating profile of yeasts strains isolated from *Rabilé* (Gallery API 20C AUX results).

Parameters	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Total (%)
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Glycerol	−	−	+	−	−	−	−	−	−	−	+	−	−	−	14
Calcium 2-keto-Gluconate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0
L-arabinose	+	−	+	−	−	−	−	−	−	−	−	+	+	−	28
D-Xylose	+	−	−	−	−	−	−	−	−	−	−	+	−	+	24
Adonitol	+	−	−	−	−	−	−	−	−	−	−	−	−	−	8
Xylitol	+	−	−	−	−	−	−	−	+	−	+	−	−	−	18
D-galactose	+	+	+	+	−	−	−	+	−	−	+	+	+	+	74
Inositol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0
D-sorbitol	+	−	+	−	−	−	+	−	−	+	+	−	+	−	40
Methyl-*α* D-Glucopyranoside	−	−	−	+	−	−	−	−	−	+	−	−	−	−	20
N-acetyl-glucosamine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0
D-cellobiose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0
D-lactose	−	−	+	−	−	+	+	−	−	−	−	−	−	−	12
D-maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
D-Saccharose	+	+	+	+	−	+	+	+	−	−	−	−	+	+	66
D-trehalose	−	−	+	−	−	−	+	+	−	+	−	+	+	−	28
D-melezitose	−	−	+	−	−	−	−	+	−	−	−	−	−	−	10
D-raffinose	+	−	−	−	−	−	−	+	−	+	−	+	+	−	30
Numbers of isolates (%)	4	9	3	5	4	1	2	2	1	5	4	5	2	3	

A: Tk2-9, BB1-9, BB3-3, BB3-1; **B:** Fd2-2, Og3-4, Ga3-4, Bf1-9, Dd2-8, Tk3-8, Oy3-8, Ga3-7, Tk2-6; **C**: Oy3-8, BB1-6, Tk3-7; **D**: Oy2-9, Ga2-5, Dr1-9, BB2-1, Ga2-3; **E**: Ga3-10, Og3-2, Ga3-3, Kb3-3, **F**: Bf2-7; **G**: Kb2-9, Og2-3; **H:** Dr1-6, Ga2-1; **I**: Oy3-9; **J**: Fd2-10, Oy3-10, Ga2-5, Dr1-9, Oy1-5; **K**: Bf2-2, Oy2-5, Bf1-8, Bf2-3; **L**: BB1-7, Bf3-4, Oy3-3, BB1-1, Dr1-6; **M**: Oy3-3, Bf2-6; **N**: Og2-9, Bf2-5, Bf1-1; (+) Assimilated; (−) Not assimilated.

The results in Table 4 show that the majority of yeasts strains isolated from Rabilé fermented glucose, maltose, sucrose, galactose and raffinose whereas none fermented lactose. The nitrogenous sources assimilation test show that a majority of yeasts strains isolated from Rabilé assimilated L-lysine, cadaverine and sulfate of ammonium, whereas a minority assimilated nitrate. The diazonium blue B test revealed that 8% of the isolates were basidiomycetous whereas 92% were ascomycetous. According to their fermentation profile and the nitrogen assimilation pattern, the fifty yeasts strains could be grouped into 18 distinct groups. 18% were in the first group, 14% were in the second group, 10% in the third group and the rest are distributed in the other 11 groups.

According to the phenotypic characteristics the fifty yeasts strains isolated were found to belong to four genera and seven species of yeasts. These are *Saccharomyces cerevisiae* (64%), *Rhodotorula muciloginosa,* (8%), *Candida pseudorhangii* (6%), *Candida heliconiae* (12%), *Candida utilis* (4%), *Shizosaccharomyces pombe* (4%) and *Sporobolomyces odoratus* (2%). *Saccharomyces cerevisiae* was found to be the dominant yeast species in the *Rabilé*.

Table 4. Carbon sources fermentation and nitrogen sources assimilation by yeasts strains isolated from *Rabilé*.

Groups	Strains isolated	Fermentation						Assimilation of nitrogen source					DBB Test[2]
		Glu	Mal	Gal	Lac	Suc	Raf	Nit	Eth	Lys	Cad	SAM	
I	Fd2-2, Dd2-8, Tk3-8, Oy3-8, Ga3-7, Tk2-6, Fd2-10, Oy3-10, Ga2-5	+	+	+	−	+	+	−	+	+	+	+	−
II	Oy3-3, Bf2-6, Og3-4, Ga3-4, Dr1-9, Oy1-5, Bf1-9	+	+	+	−	+	+	−	−	+	+	+	−
III	Oy2-9, Ga2-5, Dr1-9, BB2-1, Ga2-3	+	+	+	−	+	−	+	+	−	−	−	−
IV	BB1-7, Bf3-4, Oy3-3, BB1-1, Dr1-6	+	+	−	−	+	+	−	−	+	−	−	−
V	Bf2-2, Oy2-5, Bf1-8, Bf2-3	+	+	−	−	+	−	+	−	−	+	+	−
VI	Dr1-6, Ga2-1, Oy3-9	+	−	−	−	+	+	−	+	−	+	+	−
VII	Tk2-9, BB1-9, BB3-1	−	−	−	−	−	−	+	+	+	+	+	+
VIII	Og 2-9, Bf 2-5, Bf 1-1	+	−	−	−	+	−	−	−	−	+	+	−
IX	Oy3-9, Kb2-9, Og2-3	+	−	+	−	+	−	−	−	−	+	+	−
X	BB1-6, Tk3-7	+	+	+	−	−	+	−	−	−	−	+	−
XI	Ga3-10, Ga3-3	+	−	−	−	−	−	+	−	−	+	−	−
XII	Og3-2, Kb3-3	+	+	−	−	−	−	−	+	−	+	−	−
XIII	BB3-3	−	−	−	−	−	−	+	−	+	+	−	+
XIV	Bf2-7	+	−	−	−	−	−	−	+	+	−	+	−
	Frequency (%)	92	68	52	0	78	64	30	46	52	74	70	8

Glu: Glucose; Suc: Sucrose; Lac: Lactose; Gal: Galactose; Mal: Maltose, Raf: Raffinose, Nit: Nitrate; Lys: Lysine; Eth: Ethylamine; Cad: Cadaverine; SAM: Sulfate of Ammonium; [2]DBB = Biazonium Blue B; (+) Fermented or assimilated; (−) Did not fermented or did not assimilated.

The diversity of yeasts strains contained in *Rabilé* can be explained by the fact African traditional alcoholic beverage production results from spontaneous fermentation and as a result, both desirable and non-desirable strains are present in the product [16]. *Saccharomyces cerevisiae* appeared in this study to be the most predominant yeast strain in *Rabilé* (64%) (**Figure 2**), a traditional fermented beer starter using as condiment in Burkina Faso. These findings are in accordance with previous studies reported by other authors. Konlani [8] found that *Saccharomyces cerevisiae* accounted for 55% - 90% of yeast population in samples of sorghum beer originated from Togo and Burkina Faso. N'guessan [17] found that *Saccharomyces cerevisiae* is the most representative yeast strain (87.36%) associated with *tchapalo* fermentation in Ivory Coast. Similarly, [15] in Ghana found that the majority of 180 yeasts strains isolated from *burukutu* were *Saccharomyces cerevisiae*. Also, Djegui [1] and Djêgui [6] found a higher frequency of *Saccharomyces cerevisiae* respectively at 54% and 71% from *otché* and *kpete-kpete*, traditional starters of Benin opaque sorghum beer. According to [18] to be accepted as *Saccharomyces cerevisiae*, the yeast strain should be able to assimilate glucose, sucrose, maltose, raffinose and ethanol. But in this study, some of the isolates classified as *Saccharomyces cerevisiae* could not assimilate all of these sugars. Similar results were found by [1] [6] [19] with yeasts

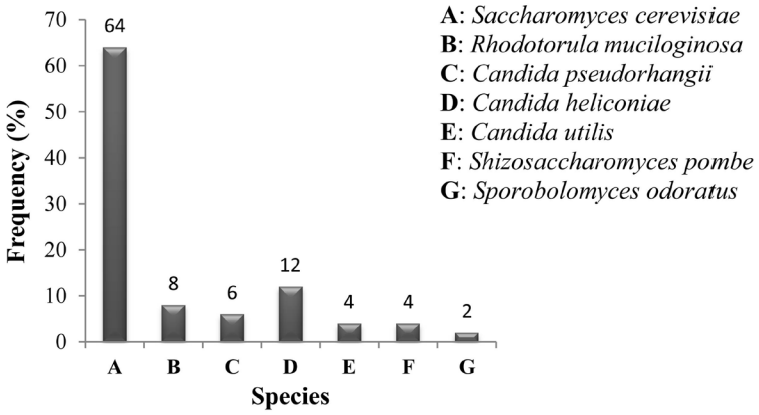

Figure 2. Frequency distribution of yeast species contained in the *Rabilé*.

strains identified as *Saccharomyces cerevisiae* but that could not assimilate all of those sugars. The identification of yeasts from *Rabilé* shows that the consumption of *Rabilé* could cause food intoxication. If *Saccharomyces cerevisiae* and *Candida utilis* have been approved for use as additive by Food and Drug Administration [20], it is not the case for *Rhodotorula muciloginosa*, *Candida pseudorhangii*, *Candida heliconiae*, *Shizosaccharomyces pombe* and *Sporobolomyces odoratus* even if they are used to produce value-added products.

4. Conclusion

This study on characterization and identification of yeasts isolated from *Rablilé* showed that *Saccharomyces cerevisiae* is the predominant specie of yeast in the traditional starter *Rabilé* used as condiment in Burkina Faso. Moreover, for more robust microbial identification, we suggest another approach based on genome fingerprinting techniques for the characterization of traditional starter *Rabilé* used as condiment. Such an approach would lead to an improvement of *Rabilé* quality and also avoid food intoxication that *Rabilé* consumption can induce.

Acknowledgements

The authors wish to thank the Germany DAAD service for the financial support to one of the authors (MOGMENGA Iliassou) through it In Region/In country scholarship program at the University of Nigeria Nsukka.

Conflicts of Interest

The author(s) have not declared any conflict of interests.

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