

# Characteristics and Diversity of Yeast in Locally Fermented Beverages Sold in Nigeria

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## ABSTRACT [ENGLISH/ANGLAIS]

Yeasts play a central role in the fermentation of foods and beverages, mainly those with high carbohydrate content which can survive and grow under stress conditions. Fermented beverages (burukutu, pito and palmwine) were selected in order to characterize the indigenous yeast flora by API AUX 20C kit. The prevalence rate of different yeast strains isolated in the research shows that yeast varies in their morphological and physiological characteristics. The prevalence rate of the yeast strains with respect to their sources of isolation are; burukutu (39.29%), pito (33.93%) and palmwine (26.79%) respectively. The yeast strains isolated are *Saccharomyces cerevisiae* 1, *Saccharomyces cerevisiae* 2, *Rhodotorula mucilaginosa* 2, *Candida colliculosa*, *Candida utilis*, *Candida magnolia*, *Rhodotorula mucilaginosa* 1, *Trichosporon asahii*, *Rhodotorula glutinis*, *Candida pelliculosa* and *Cryptococcus albidus*

**Keywords:** *Saccharomyces cerevisiae*, fermented beverages, yeast, morphology, assimilation

## RÉSUMÉ [FRANÇAIS/FRENCH]

Les levures jouent un rôle central dans la fermentation des aliments et des boissons, surtout celles avec un contenu riche en glucides qui peuvent survivre et se développer dans des conditions de stress. Boissons fermentées (burukutu, pito et vin de palme) ont été sélectionnés afin de caractériser la flore de levures indigènes par kit API 20C AUX. Le taux de prévalence de différentes souches de levure isolées dans la recherche montre que la levure varie dans leurs caractéristiques morphologiques et physiologiques. Le taux de prévalence des souches de levures à l'égard de leurs sources d'isolement sont; burukutu (39,29%), pito (33,93%) et vin de palme (26,79%) respectivement. Les souches de levure *Saccharomyces cerevisiae* isolée sont 1, 2 *Saccharomyces cerevisiae*, *Rhodotorula mucilaginosa* 2, *Candida colliculosa*, *Candida utilis*, *Candida magnolia*, *Rhodotorula mucilaginosa* 1, *Trichosporon asahii*, *Rhodotorula glutinis*, *Candida* et *Cryptococcus albidus pelliculosa*

**Mots-clés:** *Saccharomyces cerevisiae*, boissons fermentées, la levure, la morphologie, l'assimilation

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## INTRODUCTION

Fermentation processes play important roles in food technology in developing countries. In traditional fermentation processes, natural micro-organisms are employed in the preparation and preservation of different types of food. These processes add to the nutritive value of foods as well as enhancing flavour and other desirable qualities associated with digestibility and edibility [1]. Nigeria is endowed with a wide range of fermentable indigenous staple foods that serve as raw materials for agro-allied cottage industries. These industries utilize small-scale equipment and provide alternative equipment for rural communities while adding value to such local produce [1]. Traditional or natural fermentation methods are initiated by

endogenous flora to yield products that have unique or single quality attributes [2]. Many European wines are made via natural fermentations relying on the naturally occurring yeast present on the grape surface and winery equipment which is efficient in a small scale basis given the inherent flexibility in terms of quality and time [3]. In contrast, large scale fermentations of food and beverages demands consistent production quality and predictable production schedules, as well as stringent quality control to ensure food safety [4]. Modern fermentations are initiated by starter cultures consisting of microorganisms that are inoculated directly into food materials to overwhelm the existing flora and bring about desired changes in the finished product. Changes may include novel functionality, enhanced preservation, reduced food

safety risks, improved nutritional or health value, enhanced sensory qualities, and increased economic value [1].

The fermented foods in Nigeria are classified into groups according to the substrates or raw materials employed. These include tubers (cassava products: 'gari', 'lafun', 'fufu'), Cereals (maize, sorghum, millet: ogi, pito, burukutu), legumes (locustbeans, soybeans: iru, dawadawa), fruit (melon: ogiri), beverages (palm wine) and animal proteins (milk: cheese). The microflora involved in the fermentation of these foods has been reported by Oyewole and Odunfa [5]. Good-quality raw materials that have been efficiently graded and sorted, simple equipment, optimum conditions, and attractive packaging are the key requirements of a food fermentation industry [5]. The aim of this research work is to isolate and characterize the indigenous yeast associated with some selected fermented beverages.

## MATERIALS AND METHODS

### Collection of Samples

Fermented beverages (burukutu, pito and palmwine) were purchased from sellers in stores and hawkers within Nigeria.

### Isolation of Yeast Strains

Serial dilution ( $10^{-1}$  to  $10^{-6}$ ) of the samples were prepared using peptone water as diluent and 0.1 ml of each sample was inoculated on yeast peptone dextrose agar (yeast extract 10 g; glucose, 10 g; agar, 15 g; peptone, 20 g; distilled water, 1000 ml) containing 50 µg chloramphenicol/ml [6] using spread plate method and the agar plates were incubated at 30 °C for 48 h.

### Yeast Identification

The yeast strains were identified according to the method of Jimoh [7] with some modifications. Morphological and physiological characteristics (such as surface characteristics, presences of pseudohyphae, ascospore formation and vegetative reproduction) of the yeast isolates were determined respectively. The yeast isolates were characterized to the species level using API 20 C AUX kit (BIOMÉRIEUX)

## RESULTS

### Characterization of Yeast Isolates Obtained From Fermented Beverages

A total of 56 yeast isolates were selected based on their morphological characteristics and were identified to the

species level using API 20 C AUX Kit (BIOMÉRIEUX), these include *Candida colliculosa*, *Candida utilis*, *Candida magnolia*, *Candida pelliculosa*, *Cryptococcus albidus*, *Rhodotorula glutinis*, *Rhodotorula mucilaginosa 2*, *Rhodotorula mucilaginosa 1*, *Saccharomyces cerevisiae 1*, *Saccharomyces cerevisiae 2* and *Trichosporon asahii* respectively. Morphological characteristics such as colonial morphology (colony shape, colour, and surface appearance), vegetative morphology (cell shape and arrangement), presence of pseudomycelium or true mycelium, pellicle and ascospore formation are presented in table 1 while the prevalence rate of yeast strains with respect to their sources of isolation are shown in table 2 respectively. The carbon assimilation pattern of the yeast isolates is as shown on table 3.

## DISCUSSION

The diversity of yeast in locally fermented beverages utilized for the research showed that most traditional fermentations employ the whole range of natural microflora that could function under the varied environmental and non-sterile conditions presented by the different processes (Tables 1 and 2). Carbon assimilation is an important criterion in the taxonomy and identification of yeasts which depends on organic carbon sources for their energy supply and growth (Table 3). Galactose is a 'non-conventional' nutrient for yeast, which however can be used as a sole carbon source when glucose is absent from the medium. Thus, the ability of the yeast cells to assimilate galactose indicated the expression of the GAL genes [8]. Furthermore, yeast strains that metabolized sucrose, lactose, cellobiose express genes that activate the synthesis of invertase, beta-galactosidase and beta-glucosidase which eventually hydrolyse the substrates to glucose and fructose, glucose and galactose; and two glucose units respectively [9]. The inability of *S. cerevisiae* and other yeast isolated except few strains *Trichosporon asahii*, *Candida pelliculosa* and *Rhodotorula mucilaginosa 2* to metabolize xylose indicated that the strain lacks xylose reductase and xylitol dehydrogenase genes responsible for xylose-fermentation [10]. All strains of *Saccharomyces cerevisiae 1* and few strains of *Candida utilis*, *Cryptococcus albidus*, *Rhodotorula mucilaginosa 2*, *Candida magnolia*, *Rhodotorula glutinis* lacks the gene responsible for synthesis of trehalase enzyme required for breakdown of trehalose to glucose because both synthesis and degradation are regulated via cAMP [11]. Summarily, It is well established that most yeasts isolated employed

different sugars as their main carbon and hence energy source.

Furthermore, the presence of *R. glutinis*, *R. mucilaginosa 1*, and *R. mucilaginosa 2* in the fermented beverages signified cross-contamination during preparation because *Rhodotorula* species have been reported as a saprophyte from skin, vaginal and respiratory specimens [12]. Contamination may also occur through contaminated containers because all members of the *Rhodotorula* genus have affinity for synthetic materials in general [13]. The incidence of the yeast *R. mucilaginosa* in the fermented beverages is very dangerous because it has been reported to cause Onychomycosis which is a dermatological problem in immunocompetent patient [14]. Despite the detrimental effect of the genera *Rhodotorula*, they are carotenoid biosynthetic yeasts easily identifiable by distinctive cream coloured to orange, red, pink or yellow colonies [15]. The incidence of *R. glutinis* and *R. mucilaginosa* obtained in this research shows that fermented beverages can serve as sources of isolation to increase yield of these pigments and improve biomass production through strain improvement.

Presence of *Candida magnolia* in the fermented beverages is beneficial in the fermentation sector because it is

industrially important yeast with substantial erythritol-producing ability and also used in biotechnology to produce mannitol from glucose [16]. Industrially, the *C. utilis* isolates obtained in this research can be utilized for production of biomass from different culture medium and as flavouring in processed foods and pet foods. Also, *C. pelliculosa* isolated can be used for modeling growth and predicting the contamination level of fermented beverages according to the report of Tchang Tchango [17]. The presence of *C. albidus* in fermented beverages showed that there was cross-contamination during fermentation through the handlers, according to Sugita [18] which reported different sources of *C. albidus* such as nails, lungs, sputum, a beer bottle, bone, blood, pigeon excreta and soil. *Trichosporon asahii* are widespread and have been isolated from a wide range of substrates, including human hair [19]. Consumption of locally fermented beverages containing *Trichosporon asahii* can also cause severe opportunistic infections (trichosporonosis) in immunocompromised individuals [20].

**Table 1:** This table shows morphological characteristics of yeast strains isolated from fermented beverages

Colony shape and colour	Colony surface appearance	Vegetative morphology		Ascospore formation	Pellicle formation	Pseudomycelium /true mycelium	Identity
		Cell shape	Arrangement				
White to creamy	Smooth and flat	Spherical cells	Singly budding	+	+	-	<i>Candida colliculosa</i>
Creamy	Smooth and flat	Ellipsoidal cells	Multipolar budding	+	+	+	<i>Candida utilis</i>
White to creamy	Smooth and flat	Ellipsoidal cells	Singly budding	+	+	+	<i>Candida pelliculosa</i>
White to creamy	Soft and smooth	Globose to oval cells	Multipolar budding	-	-	-	<i>Candida magnolia</i>
Creamy	Smooth and flat	Spherical cells	Multipolar budding	-	+	+	<i>Cryptococcus albidus</i>
Pinkish red	Smooth and flat	Spherical cells	Multipolar budding	-	-	-	<i>Rhodotorula mucilaginosa 1</i>
Pinkish	Smooth and flat	Ellipsoidal cells	Singly budding	-	+	-	<i>Rhodotorula mucilaginosa 2</i>
Pinkish	Smooth and shiny	Spherical cells	Multipolar budding	-	-	-	<i>Rhodotorula glutinis</i>
Creamy and spherical	Smooth, shiny; flat or raised	Spherical or Elongated cells	Singly budding	+	-	-	<i>Saccharomyces cerevisiae 1</i>
Creamy and spherical	Smooth or moisten; flat or raised	Spherical, elongated or oval cells	Multipolar budding	+	-	-	<i>Saccharomyces cerevisiae 2</i>
White to creamy	Wrinkled with irregular edges	Spherical cells	Singly budding	-	+	+	<i>Trichosporon asahii</i>

+ = Ascospore, pellicle, pseudomycelium and true mycelium present; - = Ascospore, pellicle, pseudomycelium and true mycelium absent

**Table 2:** This table shows prevalence rate of Yeast strains with respect to their sources of isolation

Species	Sources of		Pito (%)	Frequencies of Isolation (%)
	Burukutu (%)	Isolation Palmwine (%)		
<i>Saccharomyces cerevisiae</i> 1	7 (12.50)	-	2 (3.57)	9 (16.07)
<i>Saccharomyces cerevisiae</i> 2	8 (14.29)	9 (16.07)	7 (12.5)	24 (42.86)
<i>Candida colliculosa</i>	-	1 (1.79)	-	1 (1.79)
<i>Candida utilis</i>	-	1 (1.79)	2 (3.57)	3 (5.36)
<i>Candida magnolia</i>	-	1 (1.79)	-	1 (1.79)
<i>Candida pelliculosa</i>	2 (3.57)	-	1 (1.79)	3 (5.36)
<i>Rhodotorula glutinis</i>	4 (7.14)	-	4 (7.14)	8 (14.29)
<i>Rhodotorula mucilaginosa</i> 1	-	1 (1.79)	-	1 (1.79)
<i>Rhodotorula mucilaginosa</i> 2	-	1 (1.79)	2 (3.57)	3 (5.36)
<i>Trichosporon asahii</i>	-	1 (1.79)	-	1 (1.79)
<i>Cryptococcus albidus</i>	1 (1.79)	-	1 (1.79)	2 (3.57)
Total number of Isolates (%)	22 (39.29)	15 (26.79)	19 (33.93)	56 (100)

**Table 3:** This table shows carbon Assimilation Test using API 20 C AUX kit (BioMEIREUX)

G	G	2	A	X	A	X	G	I	S	M	N	C	L	M	S	T	M	R	IDENTITY
L	L	K	R	Y	D	L	A	N	O	D	A	E	A	A	A	R	L	A	
U	Y	G	A	L	O	T	L	O	R	G	G	L	C	L	C	E	Z	F	
+	-	-	-	-	-	-	v	-	-	+	-	-	-	+	+	v	v	V	<i>Saccharomyces cerevisiae</i> 1
+	-	-	-	-	-	-	v	-	-	v	v	-	-	+	+	+	v	V	<i>Saccharomyces cerevisiae</i> 2
+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+	+	<i>Rhodotorula mucilaginosa</i> 1
+	+	-	-	v	v	+	+	-	-	-	v	v	v	+	+	v	+	+	<i>Rhodotorula mucilaginosa</i> 2
+	v	+	-	-	-	-	-	-	-	v	-	-	-	+	+	+	+	V	<i>Rhodotorula glutinis</i>
+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	+	+	<i>Candida utilis</i>
+	v	-	-	v	-	-	-	-	-	+	-	-	-	+	+	+	+	V	<i>Candida pelliculosa</i>
+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	<i>Candida magnolia</i>
+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	+	<i>Candida colliculosa</i>
+	-	v	-	-	-	-	-	-	-	+	-	+	-	+	+	v	+	V	<i>Cryptococcus albidus</i>
+	+	+	+	+	-	-	+	-	-	-	-	-	-	+	+	+	-	+	<i>Trichosporon asahii</i>
+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	<i>Saccharomyces pastorianos</i>

+ = Carbon assimilated (turbid) / pinkish colour in the case of *Rhodotorula* spp; - = Carbon not assimilated (non-turbid); V (Variation) = +/-; GLU = D- glucose; MDG = methyl- $\alpha$ D-glucopyranosides; GLY = Glycerol; NAG = N- acetyl-glucosamine; 2KG = 2-keto-gluconate; CEL = D- cellobiose; ARA = L-arabinose; MAL = D-Maltose; XYL = D-xylose; SAC = D-Saccharose (sucrose); ADO = Adonitol; TRE = D-Trehalose; XLT = Xylitol; MLZ = D-Melezitose; GAL = D-Galactose; RAF = D- Raffinose; INO = Inositol; LAC = D-Lactose; SOR = D-Sorbitol

## CONCLUSION

The fermented beverages are characterized by numerous microorganisms of varying functions that could be beneficial or detrimental to the fermentation processes; mixed cultures that produce the blend of rich flavours of the product and some microorganisms that would accelerate spoilage in the finished products. Many indigenous traditional technologies are not easily adopted by transnational companies without altering the methods of preparation thus; producing a product of

altered flavour and unacceptability. Since indigenous peoples are sources of traditional food systems, inter-institutional initiative will contribute to the development of these resources if indigenous people are encouraged to participate.

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#### CONFLICT OF INTEREST

No conflicts of interests were declared by authors.