Research Journal of Biological Sciences 6 (8): 389-392, 2011
ISSN: 1815-8846

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

[1]S.O. Jimoh, [2]S.A. Ado, [2]J.B. Ameh and [2]C.M.Z. Whong
[1]Department of Biological Sciences, Microbiology Unit,
Fountain University, Osogbo, Osun State, Nigeria
[2]Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Abstract: 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 associated 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*, respectively.

Key words: *Saccharomyces cerevisiae*, fermented beverages, yeast, morphology, assimilation, grow, Nigeria

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. 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. Traditional or natural fermentation methods are initiated by endogenous flora to yield products that have unique or single quality attributes (Obire, 2005).

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 (Julet, 2004).

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 (Hutkins, 2006). Modern fermentations are initiated by starter cultures consisting of micro organisms 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.

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, soyabeans: iru, dawadawa), fruit (melon:ogiri), beverages (palmwine) and animal proteins (milk:cheese). The micro-flora involved in the fermentation of these foods has been reported by Oyewole and Odunfa (1990). 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. The aim of this research 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 dilutions (10^{-1}-10^{-6}) of the samples were prepared using peptone water as diluent

Corresponding Author: S.O. Jimoh, Department of Biological Sciences, Microbiology Unit, Fountain University, Osogbo, Osun State, Nigeria

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 ug chloramphenicol mL^{-1} (Teramoto *et al*., 2005) 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 with some modifications. Morphological and physiological characteristics (such as surface characteristics, presences of pseudohyphae, ascopore 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 (BIOMERIEUX).

RESULTS AND DISCUSSION

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 (BIOMERIEUX), 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 shown 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 in Table 3. The diversity of yeast in locally fermented beverages utilized for the research showed that most traditional fermentations employ the whole range of natural micro-flora that could function under the varied environmental and non-sterile conditions presented by the different processes (Table 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

Table 1: Morphological characteristics of yeast strains isolated from fermented beverages

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

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

Table 2: Prevalence rate of yeast strains with respect to their sources of isolation

Species	Sources of isolation (%)			Frequencies of isolation (%)
	Burukutu	Palm wine	Pito	
Saccharomyces cerevisiae 1	7 (12.50)	-	2 (3.57)	9 (16.07)
Saccharomyces cerevisiae 2	8 (14.29)	9 (16.07)	7 (12.50)	24 (42.86)
Candida colliculosa	-	1 (1.79)	-	1 (1.79)
Candida utilis	-	1 (1.79)	2 (3.57)	3 (5.36)
Candida magnolia	-	1 (1.79)	-	1 (1.79)
Candida pelliculosa	2 (3.57)	-	1 (1.79)	3 (5.36)
Rhodotorula glutinis	4 (7.14)	-	4 (7.14)	8 (14.29)
Rhodotorula mucilaginosa 1	-	1 (1.79)	-	1 (1.79)
Rhodotorula mucilaginosa 2	-	1 (1.79)	2 (3.57)	3 (5.36)
Trichosporon asahii	-	1 (1.79)	-	1 (1.79)
Cryptococcus albidus	1 (1.79)	-	1 (1.79)	2 (3.57)
Total No. of isolates (%)	22 (39.29)	15 (26.79)	19 (33.93)	56 (100.00)

Table 3: Carbon assimilation test using API 20 C AUX kit (BIOMEIREUX)

	Genotypes																		
Identity	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF
Saccharomyces cerevisiae 1	+	-	-	-	-	-	-	v	-	-	+	-	-	-	+	+	v	v	v
Saccharomyces cerevisiae 2	+	-	-	-	-	-	-	v	-	-	v	v	-	-	+	+	+	v	v
Rhodotorula mucilaginosa 1	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+	+
Rhodotorula mucilaginosa 2	+	+	-	-	v	V	+	+	-	-	-	v	v	v	+	+	v	+	+
Rhodotorula glutinis	+	v	+	-	-	-	-	-	-	-	v	-	-	-	+	+	+	+	v
Candida utilis	+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	+	+
Candida pelliculosa	+	v	-	-	v	-	-	-	-	-	+	-	-	-	+	+	+	+	v
Candida magnolia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
Candida colliculosa	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	+
Cryptococcus albidus	+	-	v	-	-	-	-	-	-	-	+	-	+	-	+	+	v	+	v
Trichosporon asahii	+	+	+	+	+	-	-	+	-	-	-	-		-	+	+	+	-	+
Saccharomyces pastorianos	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+

+ = Carbon assimilated (turbid)/pinkish colour in the case of *Rhodotorula* sp.; - = Carbon not assimilated (non-turbid); V (Variation) = +/-; GLU = D-Glucose; MDG = Methyl-αD-Glucopyranosides; GLY = Glycerol, NAG = N-Acetyl-Glucosamine; 2KG = 2-Keto-Gluconat; 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

glucose is absent from the medium. Thus, the ability of the yeast cells to assimilate galactose indicated the expression of the *GAL* genes (Yun *et al*., 2001).

Furthermore, yeast strains that metabolized sucrose, lactose, cellibiose 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 (Ogawa *et al*., 2000). 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 (Kosman, 2003).

All strains of *Saccharomyces cerevisiae* 1 and few strains of *Candida utilis*, *Cryptococcus albidus*, *Rhodotorula mucilagnosa* 2, *Candida magnolia* and *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 (Versele and Thevelein, 2001). 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 (Gomez-Lopez *et al*., 2005). Contamination may also occur through contaminated containers because all members of the Rhodotorula genus have affinity for synthetic materials in general (Goyal *et al*., 2008). 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 (Cunha *et al*., 2009). Despite the dentrimental effect of the genera Rhodotorula, they are carotenoid biosynthetic yeasts easily identifiable by distinctive cream coloured to orange, red, pink or yellow colonies (Krinsky, 2001). The incidence of *R. glutinis* and *R. mucilagnosa* 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 an industrially important yeast with substantial erythritol-producing ability and also used in biotechnology to produce mannitol from glucose (Lee *et al*., 2003). 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. pellicullosa* isolated can be used for modeling growth and predicting the contamination level of fermented beverages according to the report of Tchango *et al*. (1997). The presence of *C. albidus* in fermented beverages showed that there was cross-contamination during fermentation through the handlers according to Sugita *et al*. (2001) 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 (Erer *et al*., 2000).

Consumption of locally fermented beverages containing *Trichosporon asahii* can also cause severe opportunistic infections (Trichosporonosis) in immuno-compromised individuals (Taj-Aldeen *et al*., 2009).

CONCLUSION

The fermented beverages are characterized by numerous micro-organisms 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 micro-organisms 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, interinstitutional initiative will contribute to the development of these resources if indigenous people are encouraged to participate.

REFERENCES

Cunha, da M.M.L., L.P.B.D. Santos, M.D. Ribeiro, A.B. Vermelho and S. Rozental, 2009. Identificationantifungal susceptibility and scanning electron microscopy of a keratinolytic strain of Rhodotorula mucilaginosa: A primary causative agent of onychomycosis. FEMS Immunol. Med. Microbiol., 55: 396-403.

Erer, B., M. Galimberti, G. Lucarelli, C. Giardini and P. Polchi *et al*., 2000. Trichosporon beigelii: A life-threatening pathogen in immunocompromised hosts. Bone Marrow Transplant, 25: 745-749.

Gomez-Lopez, A., E. Mellado, J.L. Rodriguez-Tudela and M. Cuenca-Estrella, 2005. Susceptibility profile of 29 clinical isolates of *Rhodotorula* spp and literature review. J. Antimicrob. Chemother., 55: 312-316.

Goyal, R., S. Das, A. Arora and A. Aggarwal, 2008. Rhodotorula mucilaginosa as a cause of persistent femoral nonunion. J. Postgrad. Med., 54: 25-27.

Hutkins, R.W., 2006. Microbiology and Technology of Fermented Foods, 1st Edn., Blackwell Publishing, Australia, Pages: 473.

Julet, M., 2004. Benefits and uses of Microorganisms. In: Introduction to Microbiology: A Case History Approach. Ingraham, J.L. and C.A. Ingraham (Eds.). Brooks/Cole-Thomson, Calif. USA., pp: 270-289.

Kosman, D.J., 2003. Molecular mechanisms of iron uptake in fungi. J. Mol. Microbiol., 47: 1185-1197.

Krinsky, N.I., 2001. Carotenoid antioxidants. J. Nutr., 17: 815-817.

Lee, J.K., B.S. Koo, S.Y. Kim and H.H. Hyun, 2003. Purification and characterization of a novel mannitol dehydrogenase from a newly isolated strain of *Candida magnoliae*. Applied Environ. Microbiol., 69: 4438-4447.

Obire, O., 2005. Activity of Zymomonas species in palm-sap obtained from three areas in Edo State, Nigeria. J. Applied Sci. Environ. Manage., 9: 25-30.

Ogawa, Y., A. Nitta, H. Uchiyama, T. Imamura, H. Shimoi and K. Ito, 2000. Tolerance mechanism of the ethanol-tolerant mutant of sake yeast. J. Biosci. Bioeng., 90: 313-320.

Oyewole, O.B. and S.A. Odunfa, 1990. Characterisation and distribution of lactic acid bacteria in cassava fermentation during fufu production. J. Applied Bacteriol., 68: 145-152.

Sugita, T., M. Takashima and R. Ikeda, 2001. Intraspecies diversity of *Cryptococcus albidus* isolated from humans as revealed by sequences of the internal transcribed spacer regions. J. Vet. Diagnostic Invest., 17: 598-600.

Taj-Aldeen, S.J., N. Al-Ansari, S. El Shafei, J.F. Meis, I. Curfs-Breuker, B. Theelen and T. Boekhout, 2009. Molecular identification and susceptibility of Trichosporon species isolated from clinical specimens in Qatar: Isolation of *Trichosporon dohaense* Taj Aldeen, Meis and Boekhout sp. nov. J. Clin. Microbiol., 47: 1791-1799.

Tchango, J., D. Watier, P. Eb, R. Tailliez, T. Njine and J.P. Hornez, 1997. Modeling growth for predicting the contamination level of guava nectar by *Candida pelliculosa* under different conditions of pH and storage temperature. J. Ind. Microbiol. Biotechnol., 18: 26-29.

Teramoto, Y., R. Sato and S. Ueda, 2005. Characteristics of fermentation yeast isolated from traditional Ethiopian honey wine, ogol. Afr. J. Biotecnol., 4: 160-163.

Versele, M. and J.M. Thevelein, 2001. Lre1 affects chitinase expression, trehalose accumulation and heat resistance through inhibition of the Cbk1 protein kinase in *Saccharomyces cerevisiae*. J. Mol. Microbiol., 41: 1311-1326.

Yun, C.W., M. Bauler, R.E. Moore, P.E. Klebba and C.C. Philpott, 2001. The role of the FRE family of plasma membrane reductases in the uptake of siderophore-iron in *Saccharomyces cerevisiae*. J. Biol. Chem., 276: 10218-10223.