CHARACTERIZATION AND TECHNOLOGICAL PROPERTIES OF YEASTS ISOLATED FROM RETTING CASSAVA

Sandra I. **Aghemwenhio** [1,2], Kola **Banwo** [2], Omotade R. **Ogunremi***[3] and Abiodun I. **Sanni** [2]
[1] Department of Biological Sciences, Federal University Dutsinma, Katsina State, Nigeria
[2] Department of Microbiology, University of Ibadan, Ibadan, Nigeria
[3] Department of Biological Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria
*Email: tadeogunremi@yahoo.com

Abstract
Yeasts are predominant microorganisms that play important roles during cassava fermentation. Most efforts to develop starter culture for cassava-based fermented products have been unsuccessful because of the little attention given to evaluating the technological potentials of yeasts as co-culture involved in the fermentation process. pH and total titratable acidity (TTA) of retting cassava samples were determined over a 72-hour fermentation period. Yeasts isolated from the retting cassava samples were characterized using phenotypic methods and assessed for technological properties, including extracellular enzymes (amylase, protease, lipase and pectinase) production and susceptibility pattern to antifungal agents by in vitro methods. Simultaneous decrease and increase in pH and total total titratable acidity respectively was observed over the 72 hours fermentation period. Four yeast species, namely Saccharomyces cerevisiae (44%), Candida krusei (22%), C. parapsilosis (17%) and C. tropicalis (17%) were isolated from retting cassava. High amylase production was observed for all the yeast isolates. About 94.55% of the yeast strains produced protease at varying concentrations. The yeast strains were susceptible to voriconazole and nystatin with the exception of 5 strains which were resistant to nystatin. In conclusion, selected yeast strains possess properties that will aid their selection and application in a controlled process to valorize cassava roots for food use.

Keywords: Yeasts, Enzymes, Antifungal compounds, Fermentation, Cassava

Submitted: 24.03.2017 Reviewed: 09.05.2017 Accepted: 19.06.2017

1. INTRODUCTION

A wide physiological diversity of yeasts and lactic acid bacteria (LAB) play important roles in the traditional fermentation of several plant derived substrates. During fermentation, yeasts enhance safety and improve the stability, nutritional value and organoleptic properties of foods (Jespersen, 2003). Traditional fermented foods enjoy wide acceptability among African population and play a predominant role in their diet (Holzapfel, 2002; Jespersen, 2003). However, the spontaneous nature of the fermentation processes, whereby the metabolic activities of the best adapted strains from a contaminating mix of LAB and yeasts determines the quality of the product has been implicated in the inconsistencies of product quality and the high risk of product failure (Holzapfel, 2002). The optimization of food fermentation processes to derive products with predictable and desirable qualities requires the careful selection and use of multifunctional strains of microorganisms as starter culture (Jespersen, 2003).

Cassava (*Manihot esculenta*) is the most important root crop in tropical countries (Tamang, 2010), especially Nigeria as a source of calorie. However, it is low in energy density and protein content, high in cyanide and anti-nutrient levels and prone to rapid deterioration (FAO, 1984; Kimaryo *et al.*, 2000; Kostinek *et al.*, 2007; Hellstrom *et al.*, 2010). In Sub-Saharan Africa, the tuber is traditionally fermented to mitigate these limitations and valorized into respective products such as *gari*, *fufu, agbelima, chikawgue*, *kivunde*, *kocho* and *togwa* (Oyewole, 2001; Kimaryo *et al.*, 2000; Olasupo, 2006; Hellstrom *et al.*, 2010; Tamang, 2010). The levels of cyanogenic glucosides and enteric pathogens in some of these spontaneously fermented products remain unacceptable (Kimaryo *et al.*, 2000).

Most efforts to develop starter culture to optimize the fermentation of cassava have been directed towards the selection and use of multifunctional strains of LAB. Kimaryo *et al.*

(2000) and Kostinek *et al.* (2007) have successfully selected superior LAB strains from the microflora involved in the spontaneous fermentation of cassava. However, the use of single-species starter culture for the production of traditional fermented foods has not been successful because traditional fermented foods are generated from the activities of a complex microflora (Tamang, 2010). In Kimaryo *et al.* (2000), the *Lb. plantarum* starter culture fermentation process was longer and the product was without the characteristic attributes compared to the spontaneous fermentation. The protein content of *Lb. plantarum*-fermented cassava was lower than a spontaneously fermented cassava sample (Sobowale *et al.*, 2007). Navarrete-Bolanos (2012) recommended that the use of a mixed microflora of the species that desirably impart the fermentation processes will be suitable as starter culture to produce wholesome and acceptable products.

Several species of yeasts have been described as predominant microflora in cassava fermentation (Oyewole, 2001; Oguntoyinbo, 2008; Hellstrom *et al.*, 2010). The flavor-active volatile compounds generated by yeast metabolism impart the characteristic taste and aroma of fermented cassava products (Okafor and Ejiofor, 1990). Enzymes from cassava yeasts contribute to retting and breakdown complex compounds in cassava to simpler compounds that can constitute nutrients for other microbial species (Oyewole, 2001). Yeasts have the potentials to improve the nutritional value of cassava, due to their ability to secrete vitamins and phytase (Hjortmo *et al.*, 2005; Hellström *et al.*, 2010).

Despite the predominance and importance of yeasts during cassava fermentation, there is little or no information on the starter culture potentials of yeasts isolated from fermented cassava products. A screening of yeast species present in retting cassava may reveal novel multifunctional strains that will serve as starter/adjunct culture to produce wholesome and acceptable cassava products. In this paper, yeasts were isolated from retting cassava and evaluated for their technological properties.

2. MATERIALS AND METHODS

Sample collection

Samples of fresh cassava with the fermenting water at the initial stage of retting were collected from two clusters of indigenous producers of *fufu*. Two samples each were collected from the clusters in Samonda and Ojoo both in Ibadan, South-West Nigeria. The samples were aseptically collected in sterile containers and transferred to the laboratory for physicochemical and microbial analysis over a period of 3 days.

Determination of pH and total titratable acidity (TTA)

The pH of aliquot from the retting cassava roots samples was determined as percentage lactic acid by using the method of Oyewole and Odunfa (1990). The TTA was determined by titrating 5 ml aliquot of each sample with 0.1 M of NaOH using 2 drops of phenolphthalein as indicator. The formula below was used to calculate (A.O.A.C., 1990). Samples were taken in triplicates at 24-hourly interval over a period of 3 days for both determinations.

$$\text{Total titratable acidity (\%)} = \frac{V \mathbf{x} M \mathrm{x} 0.009}{Vs} \mathbf{x100}$$

Where, V=Volume of NaOH used; M= Molarity of NaOH used; 0.009= milliequivalent factor for lactic acid; Vs= Volume of sample

Isolation and characterization of yeasts

Yeats isolation

One ml of aliquot from the samples was serially diluted using sterile distilled water. One ml of appropriate serial dilution was pour-plated in Malt Extract Agar (MEA) supplemented with 0.01mg /ml of streptomycin. Incubation was done at 35^{o}C for 48-72 h. Distinct colonies were selected from the culture plates and purified by repeated streaking on MEA plates. The pure cultures were preserved on MEA slants at 4^{o}C.

Characterization and identification of yeast isolates

Key features of yeast colonies were examined on MEA and cell morphology was determined

after lactophenol cotton blue staining and examined by using X40 objective lens of a compound microscope. Biochemical characterization was carried out by standard taxonomical methods (Kurtzman *et al.*, 2011). The biochemical tests carried out include urea hydrolysis, acid production from glucose, tolerance to 1% acetic acid and fermentation of different sugars. The yeast isolates was identified by comparing with already described yeasts in Kurtzman *et al.* (2011).

Determination of technological properties of yeast isolates

Screening of by yeast isolates for extracellular enzyme production

Preparation of yeast inoculum

A suspension of 24 h old broth culture of each yeast isolates was prepared. The suspension was standardized to 10^6 CFU/ml and inoculated on the surface of appropriate agar using multipoint inoculator.

Production of amylase

Amylase production was determined by using starch agar (per litre; 3g Beef extract, 10g soluble starch, 12g Agar). The yeast isolates were inoculated on the surface of the solidified starch agar medium and incubated at 30^0C for 48 hours. Plates were then flooded with Gram's iodine. Clear zones around regions of growth indicate hydrolysis of starch, while the unhydrolyzed starch appears as blue-black coloration (Harrigan and MaCance, 1966). Diameters of clear zones were measured (Ong *et al.*, 2011).

Production of protease

Protease production was determined on modified MEA (per litre; 50g MEA, 100g skimmed milk). The modified MEA plates were inoculated with yeast isolates and incubated at 30°C for 3 days. Uninoculaed plates served as control. Clear zone around the growth area indicate protease production. Positive result was confirmed by flooding plates with mercury chloride solution (Harrigan and MaCance, 1966). Diameters of clear zones were measured (Ong *et al.*, 2011).

Production of lipase

Lipase production was assessed on a chemically defined medium (per litre; 20g olive oil, 2.5g K_2HPO_4, 1.3g $(NH_4)_2$ SO_4, 0.5g $MgSO_4$, 0.5g yeast extract, 6.5ml urea, 20g agar, aniline blue indicator). The medium plates were inoculated with yeast isolates and incubated at 30°C for 24 h. Zone of clearance around isolate indicated that they possessed lipolytic characteristics (Kambiz *et. al.*, 2008). Diameters of clear zones were measured (Ong *et al*., 2011).

Production of pectinase

Each of the isolated yeast was screened for pectinase production using pectin agar (per litre of phosphate buffer; 0.5g $CuSO_4$,$5H_2O$, 0.5g KCl, 0.01g $MnSO_4.7H_20$, 3g $FeSO_4.7H_2O$, 1g $NaNO_3$, 0.5g $ZnSO_4$, 10g pectin, 15g agar; pH 6.2). The agar was inoculated with the yeast isolates followed by incubation at 30°C for 5 days. After incubation the plate were then flooded with Gram's iodine. Clear zones around a region of growth indicate hydrolysis of pectin, while the unhydrolyzed pectin appears as blue-black coloration (Naga and Divakar, 2013). Diameters of clear zones were measured in millimeter (Ong *et al.*, 2011).

Determination of the susceptibility pattern of yeast isolates to antimicrobials

Preparation of inoculum

Yeast isolates were inoculated into sterile Malt Extract Broth and incubated for 24 h. The broth culture was standardized with 0.5 McFarland standard. Sterile cotton swab was dipped into standardized broth culture and used to inoculate antimicrobial sensitivity plates.

Antifungal and antibacterial susceptibility pattern

Antifungal and antibacterial susceptibility patterns were respectively determined by agar overlay diffusion method. The antifungal discs include; 25µg Fluconazole, 1µg Voriconazole,

50µg Nystatin. The following antibacterial discs were used; 10µg Erythromycin, 30µg Augmentin, 10µg Gentamicin, 10µg Ofloxacin, 10µg Cloxacillin, 30µg Cefuroxime, 30µg Ceftazidine and 30µg Ceftriazone. The soaked cotton swab was used to spread the yeast over the entire surface of a solidified MEA plate and discs were aseptically and gently placed on the agar surface. The plates were incubated in inverted position at 30 ^{o}C for 24 h. The zones of clearance around the discs were examined and measured (John *et al.*, 1999). Values obtained were interpreted as either susceptible (S), intermediate (I) or resistant (R) by using a zone diameter interpretative chart for respective antifungal agent (NCCLS 2004; CLSI 2012).

3. RESULTS AND DISCUSSION

During the 72 h of retting, decrease in pH and increase in total titratable acidity of the four cassava samples was observed (Table 1). The pH of the samples ranged from 3.9 to 4.1 at the beginning of the retting process (0 h) while at the end of 72 h retting the pH had significantly changed to between 3.2 and 3.4. There was a general increase in total titratable acidity over the 72 h retting period with sample JE4 showing the most significant change from 0.24 to 0.88%. A similar observation was made by Oyewole (2001) and Ogunnaike *et al.* (2013). During cassava fermentation, LAB are mainly responsible for the release and accumulation of organic acid, resulting in lowered pH. Reduced pH improves the stability and safety of foods (Caplice and Fitzgerald, 1999) and the organic acids are carbon sources for yeast growth (Walker, 2009).

A total of eighteen yeasts were isolated from MEA plates at 24, 48 and 72 h, during the retting of pieces of cassava roots. The isolates were cream or white colonies, with spherical or ovoid cells. All of the isolates did not hydrolyse urea, fermented glucose, fructose and maltose but failed to ferment lactose, mannitol and sorbitol.

Table 1: Changes in pH and total titratable acidity of retting cassava samples

Time (h)	pH				Total titratable acidity (%)			
	SA1	SA2	JE3	JE4	SA1	SA2	JE3	JE4
0	4.1	4.0	4.1	3.9	0.28	0.28	0.28	0.24
24	3.9	3.8	3.9	3.7	0.31	0.30	0.32	0.31
48	3.7	3.6	3.6	3.5	0.69	0.62	0.65	0.60
72	3.3	3.2	3.4	3.3	0.87	0.87	0.86	0.88

Key- SA: Samonda, JE: Jerusalem

The most frequently occurring (44%) yeast isolate were presumptively characterized as *Saccharomyces cerevisiae*. Other probably identified species include *Candida krusei* (22%), *C. parapsilosis* (17%) and *C. tropicalis* (17%) (Table 2). The wide taxonomical and physiological diversity of microorganisms in traditional fermented food substrates makes them flourishing sources of technologically important microbial strains (Franz *et al.*, 2014). Yeasts are microorganisms that are significantly involved in the fermentation of most African traditional fermented foods but their roles are barely investigated (Jespersen, 2003).

These species have been widely reported in varieties of traditional fermented foods, including cassava-based products such as *agbelima, fufu, gari, kivunde, lafun, togwa* and cassava starch (Oyewole, 2001; Lacerda *et al.*, 2005; Oguntoyinbo, 2008; Hellstrom *et al.*, 2010). The predominance of *S. cerevisiae* among the yeast species in this study confirms the view of Jespersen (2003) that *S. cerevisiae* is a predominant yeast species in African fermented non-alcoholic starchy foods. The relative diversity of the species of *Candida* also agrees with the studies of Oguntoyinbo (2008) that revealed the presence of *C. tropicalis, C. rugopelliculosa, C. inconspicua* and *C. krusei* during cassava fermentation for *gari* production. *C. tropicalis* was reported in cassava fermentation for *lafun*, *fufu* and *attiéké* production (Nwachukwu and Edwards, 1987; Oyewole, 2001; Coulin *et al.*, 2006).The yeast species isolated in this study have been safely consumed in several indigenous fermented foods over decades and some are listed to have beneficial uses in foods (Hansen 2011;

Bourdichon *et al.*, 2012). *S. cerevisiae* has a Qualified Presumption of Safety status as confirmed by European Food Safety Authority (EFSA. 2007).

As shown in Table 3, all the yeast strains examined exhibited strong amylase activity with diameter of clear zones ranging between 5 and 10 mm. Eight and seven strains produced protease and lipase respectively while none produced pectinase.

Table 2: Characterization and percentage occurrence of yeasts isolated from retting cassava samples

Colony and cell morphology				
Colour	Cream	White	Cream	Cream
Elevation	Raised	Flat	Raised	Raised
Edge	Entire	Undulating	Lobed	Lobed
Cell shape	Spherical	Ovoid	Spherical	Spherical
Biochemical characteristics				
Urea hydrolysis	-	-	-	-
Acid production from glucose	+/-	-	-	+
Tolerance of 1% acetic acid	-	-	-	-
Sugar fermentation				
Glucose	+	+	+	+
Galactose	+	-	-	+
Sucrose	+	-	-	-
Lactose	-	-	-	-
Fructose	+	+	+	+
Maltose	+	+/-	+	+
Mannitol	-	-	-	-
Sorbitol	-	-	-	-
Probable identity	*Saccharomyces cerevisiae*	*Candida krusei*	*Candida parapsilosis*	*Candida tropicalis*
Percentage occurrence (%)	44	22	17	17

Key: + = positive, - = negative, +/- = variable

Table 3: Extracellular enzyme production by yeasts isolated from retting cassava

Isolates	**Diameter of zone of clearance (mm)**			
	Amylase	**Protease**	**Lipase**	**Pectinase**
S. cerevisiae RC1	++	+	-	-
C. krusei RC2	++	-	-	-
S. cerevisiae RC3	++	+	-	-
S. cerevisiae RC4	++	-	-	-
C. parapsilosis RC5	++	-	+	-
S. cerevisiae RC6	++	+	-	-
C. tropicalis RC7	++	++	-	-
C. krusei RC8	++	-	-	-
S. cerevisiae RC9	++	+	-	-
C. tropicalis RC10	++	+	+	-
C. krusei RC11	++	-	-	-
C. tropicalis RC12	++	+	-	-
S. cerevisiae RC13	++	+	-	-
C. parapsilosis RC14	++	-	+	-
C. krusei RC15	++	-	-	-
S. cerevisiae RC16	++	-	-	-
C. parapsilosis RC17	++	-	-	-
S. cerevisiae RC18	++	-	++	-

Key: '–' = no clear zone (enzyme not produced), '+' = clear zone < 5 mm (enzyme produced) and '++' = clear zone > 5 mm (enzyme highly produced)

C. tropicalis RC10 produced amylase, protease and lipase. Protease activity was not observed in strains of *C. krusei* and *C. parapsilosis.* This study revealed the potentials of the yeast isolates to produce enzymes that are of importance during food fermentation. The production of extracellular amylase by all the yeast strains could be a reflection of the abundance of starch in cassava and the adaptation of associated yeasts (FAO, 1984). A wide distribution of amylase production among yeast species isolated from retting cassava was earlier reported by Oyewole (2001). Yeasts associated with maize fermentation produced extracellular amylase (Omemu *et al.,* 2007). Yeast amylase in cassava may contribute to the breakdown of cassava starch to simple sugar, thereby increasing the energy density of cassava and providing carbon source for LAB in view of the fact that amylase production is a rare trait among LAB isolated from starchy foods (Sanni *et al.,* 2002; Kostinek *et al.,* 2007; Ogunremi and Sanni, 2011). Protease and lipase activities were relatively weak and not well distributed among the yeast isolates. Yeasts from starchy sources were previously reported to be weak in the activities of protease (Omemu *et al.,* 2007; Ogunremi *et al.,* 2015). Lipase hydrolyzes lipids in foods to produce flavor compounds and precursors of flavor compounds (Steinkraus, 1997; Arroyo-Lopez *et al.,* 2012). The few yeasts strains that showed lipase activity in this study have the potentials to impart the aromatic profile of retted cassava products. Pectinases are a group of tissue (pectin) degrading enzymes that contribute to the softening of cassava during retting (Ampe *et al.,* 1995). Our study showed that no yeast strain produced pectinase. It was earlier suggested that the softening of cassava is mediated by bacterial activity (Ampe *et al.,* 1995). However, yeast species isolated during cassava retting showed polygalacturonase activity (Oyewole, 2001).

The effects of fluconazole and nystatin on the yeasts isolates were strain-specific. Out of the eight *S. cerevisiae* and three *C. parapsilosis* strains, four and two strains were susceptible to fluconazole respectively. None of the *C. tropicalis* strains was susceptible and at least one strain of *S. cerevisiae, C. parapsilosis* and *C. tropicalis* showed intermediate susceptibility to fluconazole. All the four *C. kusei* were observed to be resistant to fluconazole. Seven *S. cerevisiae* isolates were susceptible to nystatin and all the yeasts were susceptible to voriconazole (Table 4). All the yeast isolates showed resistance to the following antibiotics; erythromycin, augmentin, gentamicin, ofloxacin, cloxacillin, cefuroxime, ceftazidine and ceftriazone (results no shown). The resistance shown by all the yeast strains to antibiotics further confirms the natural resistance of yeasts to antibacterial and these is particularly desirable because this attribute in yeasts is not mediated by mobile genetic elements (Czerucka *et al.,* 2007; Kourelis *et al.,* 2010). Hence, they do not constitute public health concerns. The emergence of new fungal infections and rising incidence of resistance of fungi to antifungal agents has made the evaluation of foodborne yeasts for resistance to antifungal compounds to be of utmost importance (Chakrabarti, 2011). In this study, the effect of antifungal agents varied from resistant, intermediate to susceptible based on the yeast strains. Broad spectrum of antifungal activity was exhibited by voriconazole and nystatin. Voriconazole is a second generation broad spectrum triazole (Donnely *et al.,* 2004; Vandeputte *et al.,* 2012). Food borne yeasts are generally not of clinical or veterinary importance (Jacques and Casaregola, 2008), however, in rare cases opportunistic infections due to antifungal resistant strains may lead to treatment failure (Ghannoum and Rice, 1999).

Table 4: *In vitro* antifungal susceptibility pattern of yeasts isolated from retting cassava

Yeast isolates	Zone of clearance (mm)		
	Fluconazole (25 µg)	Voriconazole (1 µg)	Nystatin (50 µg)
S. cerevisiae RC1	15.0 (I)	22.0 (S)	20.0 (S)
C. krusei RC2	5.0 (R)	19.0 (S)	13.0 (R)
S. cerevisiae RC3	16.0 (I)	21.0 (S)	19.0 (S)
S. cerevisiae RC4	19.0 (S)	20.0 (S)	18.0 (S)
C. parapsilosis RC5	22.0 (S)	28.0 (S)	12.0 (R)
S. cerevisiae RC6	20.0 (S)	21.0 (S)	17.0 (S)
C. tropicalis RC7	14.0 (R)	20.0 (S)	14.0 (I)
C. krusei RC8	7.0 (R)	18.0 (S)	13.0 (R)
S. cerevisiae RC9	18.0 (I)	19.0 (S)	16.0 (I)
C. tropicalis RC10	13.0 (R)	20.0 (S)	14.0 (I)
C. krusei RC11	9.0 (R)	19.0 (S)	17.0 (S)
C. tropicalis RC12	15.0 (I)	20.0 (S)	17.0 (S)
S. cerevisiae RC13	20.0 (S)	23.0 (S)	19.0 (S)
C. parapsilosis RC14	21.0 (S)	25.0 (S)	12.0 (R)
C. krusei RC15	9.0 (R)	20.0 (S)	12.0 (R)
S. cerevisiae RC16	14.0 (R)	22.0 (S)	21.0 (S)
C. parapsilosis RC17	17.0 (I)	24.0 (S)	17.0 (S)
S. cerevisiae RC18	19.0 (S)	21.0 (S)	20.0 (S)

Key: S = susceptibility, I = intermediate and R = resistance

4. CONCLUSION

In conclusion, this study revealed the predominance of *Saccharomyces cerevisiae* and species of *Candida* during cassava retting. Few of the yeast strains possess properties that will aid their selection and application in a controlled process to valorize cassava roots for production of foods with consistent and predictable quality.

5. REFERENCES

[1] A.O.A.C. (1990). Official methods of analysis. 15th edn.. Association of Official Analytical Chemists.

[2] Ampe, F., Keleke, S., Robert, H. & Brauman, A. (1995). The role and origin of pectin degrading enzymes during cassava retting. *Transformation Alimentaire du Manioc*.

[3] Arroyo-Lopez, F.N., Romero-Gil, V., Bautista-Gallego, J., Rodriguez-Gomez, F., Jimenez-Dıaz, R., Garcıa-Garcıa, P., Querol, A. &Garrido-Fernandez, A. (2012). Yeasts in table olive processing: Desirable or spoilage microorganisms? *International Journal of Food Microbiology*, 160: 42–49. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.08.003

[4] Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I.B., Prajapati, J.B., Seto, Y., Schure, E.T., Van Boven, A., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S. & Hansen, E.B.(2012). Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology*, 154: 87–97.

[5] Caplice, E. & Fitzgerald, G.F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, 50: 131–149. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.12.030

[6] Chakrabarti, A. (2011). Drug resistance in fungi–An emerging problem. *Regional Health Forum* 15(1): 97-103.

[7] Clinical and Laboratory Standard Institute (2012). Performance Standards for antimicrobial disc susceptibility test; approved standard-Eleventh edition.

[8] Coulin, P., Farah, Z., Assanso, J., Spillmann, H. & Puhan, Z. (2006). Characterisation of the microflora of attiéké, a fermented cassava product, during traditional small-scale preparation. *International Journal of Food Microbiology*, 106: 131-136. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.06.012

[9] Czerucka, D., Piche, T. & Rampal, P. (2007). Review article: yeast as probiotics – *Saccharomyces boulardii*. *Alimentary Pharmacology and Therapeutics*, 26: 767-778. http://dx.doi.org/10.1111/j.1365-2036.2007.03442.x

[10] Donnely J.P & De Pauw B.E. (2004). Voriconazole-a new therapeutic agent with an extended spectrum of antifungal activity. *Clinical Microbiology of Infections*. 10:107-112. http://dx.doi.org/10.1111/j.1470-9465.2004.00838.x

[11] European Food Safety Authority (EFSA) (2007). Opinion of the scientific committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *The European Food Safety Authority Journal*, 587: 1-16.

[12] Food and Agricultural Organization (FAO) (1984). Food and Agriculture Organization, production year Book. 38:130.

[13] Franz, C.M.A.P., Huch, M., Mathara, J.M., Abriouel, H., Benomar, N., Reid, G., Galvez, A. & Holzapfel, W.H. (2014). African fermented foods and probiotics. *International Journal of Food Microbiology*, 190: 84–96. http://dx.doi.org/10.1016/j.ijfoodmicro.2014.08.033

[14] Ghannoum, M.A. & Rice, L.B. (1999). Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clinical Microbiology Reviews*, 12(4): 501–517.

[15] Hansen, E.B. (2011). Microorganisms with technologically beneficial uses. *International Dairy Federation World Dairy Summit 2011*.

[16] Harrigan, W.F., & MaCance, M.E. (1966). The Microbiological examination of foods. In: *Laboratory Methods in Microbiology*. London: Academic press: 106-132.

[17] Hellström, A.M., Vázques-Juárez, R., Svanberg, U. & Andlid, T.A. (2010). Biodiversity and phytase capacity of yeasts isolated from Tanzanian togwa. *International Journal of Food Microbiology*, 136: 352-358. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.10.011

[18] Hjortmo, S., Patring, J., Jastrebova, J. & Andlid, T. (2005). Inherent biodiversity of folate content and composition in yeasts. *Trends in Food Science and Technology*, 16: 311–316. http://dx.doi.org/10.1016/j.tifs.2005.03.014

[19] Holzapfel, W.H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology*, 75:197– 212. http://dx.doi.org/10.1016/S0168-1605(01)00707-3

[20] Jespersen, L. (2003). Occurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African indigenous fermented foods and beverages. *FEMS Yeast Research*, 3:191–200. https://doi.org/10.1016/S1567-1356(02)00185-X

[21] Kambiz, M.H., Fereshter, E., Bagher.Y & Fatemeh. T. (2008). Isolation and identification of lipase producing *Bacillus* sp from soil. *Pakistan Journal of Biological sciences.*11 (5): 13-17.

[22] Kimaryo, V.M., Massawe, G.A., Olasupo, N.A., & Holzaphel, W.H. (2000). The use of a starter culture in the fermentation of cassava for the production of *kivunde*, a traditional Tanzanian food product. *International Journal of Food Microbiology*, 56**:** 179-190. http://dx.doi.org/10.1016/S0168-1605(00)00159-8

[23] Kostinek, M., Specht, I., Edward, V.A., Pinto, C., Egounlety, M., Sossa, C., Mbugua, S., Dortu, C., Thonart, P., Taljaard, L., Mengu, M., Franz, C.M.A.P. & Holzapfel, W.H. (2007). Charcterization and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. *International Journal of Food Microbiology*, 114:342-351. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.09.029

[24] Kourelis, A., Kotzamanidis, C., Litopoulou-Tzanetakis, E., Scourasi, Z.G., Tzanetakis, N. & Yiangou, M. (2010). Preliminary probiotic selection of dairy and human yeast strains. *Journal of Biological Research*, 13: 93–104.

[25] Kurtzman, C.P., Fell, J.W., Boekhout, T. & Robert, V. (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In: *The Yeasts, a Taxonomic Study*, C.P. Kurtzman, and J.W. Fell Eds. Elsevier, B.V, Amsterdam: 87–110.

[26] Lacerda, I.C.A., Miranda, R.L., Borelli, B.M., Nunes, A.I.C., Nardia, R.M.D., Lachance, M. & Ros, C.A. (2005). Lactic acid bacteria and yeasts associated with spontaneous fermentations during the production of sour cassava starch in Brazil. *International Journal of Food Microbiology*, 105: 213– 219. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.04.010

[27] Naga R. & G. Divakar (2013). Production of pectinase by using *Bacillus circulans* isolated from dump yards of vegetable wastes. *International Journal of Pharmaceutical Sciences and Research*. 4(7):2615-2622. http://dx.doi.org/10.13040/IJPSR.0975-8232

[28] National Committee for Clinical Laboratory Standard (2004). Methods for antifungal disc susceptibility testing of yeasts. Approved standard M44-A.

[29] Navarrete-Bola˜nos, J.L. (2012). Improving traditional fermented beverages: How to evolve from spontaneous to directed fermentation. *Engineering and Life Science*, 12(4): 1–9. http://dx.doi.org/10.1002/elsc.201100128

[30] Nwachukwu, S.U. & Edwards, A.W. (1987). Microorganism associated with cassava fermentation for lafun production. *Journal of Food and Agriculture*, 1:39-42.

[31] Ogunnaike, A.M., Adepoju, P.A., Longe, A.O., Elemo, G.N. & Oke, O.V. (2015). Effects of submerged and anaerobic fermentations on cassava flour (lafun). *Africa Journal of Biotechnology*: 961-970. http://dx.doi.org/10.5897/AJB12.25700

[32] Ogunremi, O.R. and Sanni, A.I. (2011)**.** Occurrence of amylolytic and/or bacteriocin-producing lactic acid bacteria in *ogi* and *fufu*. *Annals of Food Science and Technology*, 12, 1: 71-77.

[33] Ogunremi, O.R., Sanni, A.I. & Agrawal, R. (2015). Starter-culture and probiotic potentials of yeasts isolated from some cereal-based Nigerian traditional fermented food products. *Journal of Applied Microbiology*, 119: 797- 808. http://dx.doi.org/10.1111/jam.12875

[34] Oguntoyinbo, F.A. (2008). Evaluation of diversity of *Candida* species isolated from fermented cassava during traditional small scale gari production in Nigeria. *Food Control*, 19: 465–469. http://dx.doi.org/10.1016/j.foodcont.2007.05.010

[35] Okafor, N. & Ejiofor, A.O. (1990). Rapid detoxification of cassava mash by a yeast simultaneously producing linamarase and amylase. *Process Biochemistry International*, 25: 82-86.

[36] Olasupo, N.A. (2006). Fermentation biotechnology in Africa. *Food Biotechnology*. 2nd Edition, K. Shetty, G. Paliyath, A. Pometto and R.E. Levin Eds. CRC Press, Boca Raton.

[37] Omemu, A.M., Oyewole, O.B. & Bankole, M.O. (2007). Significance of yeasts in the fermentation of maize for ogi production. *Food Microbiology* 24: 571–576. http://dx.doi.org/10.1016/j.fm.2007.01.006

[38] Ong, K.S., Chin, H.S. & Teo, K.C. (2011). Screening of antibiotic sensitivity, antibacterial and enzymatic activities of microbes isolated from ex-tin mining lake. *Africa Journal of Microbiology Research*, 5(17): 2465. http://dx.doi.org/10.5897/AJMR11.170

[39] Oyewole, O.B. & Odunfa, S.A. (1988). Microbiological studies on cassava fermentation for 'lafun' production. *Food Microbiology*, 5: 125–133. http://dx.doi.org/10.1016/0740-0020(88)90010-X

[40] Oyewole, O.B. (2001). Characteristics and significance of yeasts' involvement in cassava fermentation for '*fufu'* production. *International Journal of Food Microbiology* 65, 213–218. http://dx.doi.org/10.1016/S0168-1605(01)00431-7

[41] Sanni, A.I., Morlon-Guyot, J. & Guyot, J.P. (2002). New efficient amylase-producing strains of *Lactobacillus plantarum* and *Lactobacillus fermentum* isolated from different Nigerian traditional fermented foods. *International Journal of Food Microbiology*, 72:53-62. http://dx.doi.org/10.1016/S0168-1605(01)00607-9

[42] Sobowale, A. O., Olurin, T. O. & Oyewole, O. B. (2007). Effect of lactic acid bacteria starter culture fermentation of cassava on chemical and sensory characteristics of fufu flour. *African Journal of Biotechnology*, 6(16): 1954-1958.

[43] Steinkraus, K.H. (1997). Classification of fermented foods: worldwide review of household fermentation techniques. *Food Control* 8 (5/6):311-317. http://dx.doi.org/10.1016/S0956-7135(97)00050-9

[44] Tamang P.J. 2010. Diversity of fermented foods. *Fermented Foods and Beverages of the World.* J.P. Tamang and K. Kailasapathy Eds. 42-84

[45] Vandeputte, P., Ferrari, S., & Alix t. Coste, A.T. (2012). Antifungal resistance and new strategies to control fungal infections. *International Journal of Microbiology*, article id 713687. http://dx.doi.org/10.1155/2012/713687

[46] Walker, G.M. (2009). Fungi | Yeasts. In: *Encyclopedia of Microbiology*, Volume 3 M. Schaechter Eds. Elsevier Inc.