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Full Length Research Paper

Categorization and segregation of yeast strains from local food crops

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Isolation and identification of yeast from *Manihot esculenta*, *Zea mays*, *Cola acuminata* and *Sorghum bicolor* was done using the spread plate technique. Morphological, cultural, physiological and molecular characterizations were carried out resulting in determination of the species. Four isolates belonging to different genera which include *Pichia, Kluyveromyces, Candida* and *Saccharomyces* were identified. This present study showed that the yeast isolates have the potential to ferment both hexose and pentose sugars.

Key words: Food crops, yeast, isolation, identification, fermentation.

INTRODUCTION

Yeasts, the chemoorganotrophs obtain carbon mostly from hexose sugars, such as glucose and fructose or disaccharides such as sucrose and maltose (Barnett, 1975). Some species can also metabolize pentose sugars like xylose (Chaudhary and Qazi, 2006), alcohols and organic acids. Yeast species either require oxygen for aerobic cellular respiration (obligate aerobes), or are anaerobic but also have aerobic methods of energy production (facultative anaerobes). The useful physiological properties of yeast have led to their use in the field of biotechnology. Fermentation of sugar by yeast is the oldest and largest application of this technology. Some isolates have the ability to carry out an alcoholic fermentation while others lack this property. Thus, many types of yeasts are used for making many foods; yeast in wine fermentation (Martini, 1992; Oelofse et al., 2008) and for xylitol production (Sreenivas Rao et al., 2004). They can also find application as biological control agents (Qing and Shiping, 2000; Chanchaichaovivat et al., 2007) and they include some of the most widely used model organisms for genetics and cell biology. Recently, the ability of yeast to convert sugar into ethanol has been harnessed by the biotechnology industry for ethanol fuel production (Oyeleke and Jibrin, 2009; Ocloo and Ayernor, 2010; Mohd et al., 2011). Thus, there is an exploration of diverse sources of yeast to seek potent species that can utilize a wide range of substrates. Kurtzman and Piskur (2006) reported that yeasts do not form a specific taxonomic or phylogenetic group and at present it is estimated that only 1% of all yeast species have been described.

In the present study, some locally available food crops were analyzed for isolation and subsequent characterization of yeast isolates which may further be

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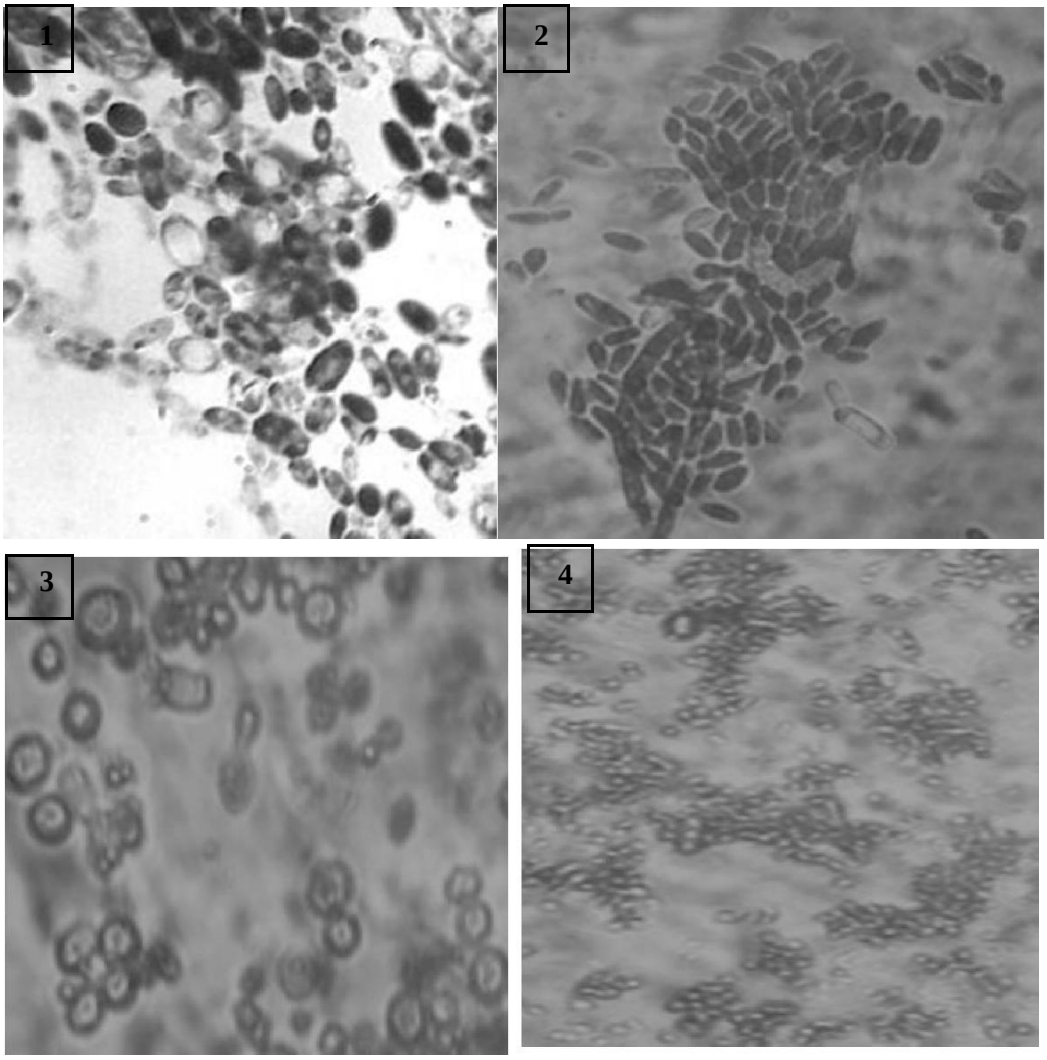

Figure 1. Microscopic characteristics of yeast strains were cultivated on MEA agar after incubation at 48 h and 28 ± 2°C 1. *K. marxianus*; 2. *C. tropicalis; 3. S. cerevisiae*; 4. *P. caribbica*

utilized in alcohol production.

MATERIALS AND METHODS

Collection of samples

Cassava tubers (*Manihot esculenta 92/0057*) were collected from the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State. Maize, guinea corn and kola nut were randomly bought from local markets within Lagos Metropolis, Nigeria. The samples were transported to the laboratory, dirt were removed, the cassava tubers were peeled. All samples were washed using sterile water and blended to powder form using a blender (Binatone). These particles were then sieved to obtain average particle sizes of 300 µm in diameter.

Isolation and identification of microorganisms

One gram of each sample was soaked in distilled water in 250 ml conical flask for 72 h at 28 ± 2°C. Serial dilution of the steep of each was carried out up to 10^{-5}. An aliquot of 0.1 ml of each dilution was plated on Malt Extract Agar (MEA) plates (5 g/100 ml) using spread plate technique. The inoculated plates were incubated for 48 h at 28 ± 2°C. Chloramphenicol at the rate of 30 µg/ml was added as an antimicrobial agent to inhibit all bacteria growth.

Subculture technique

Isolates were subculture on MEA to check for purity and incubated at 28 ± 2°C for 48 h (Figure 1). Purified cultures were routinely maintained on MEA slants and kept at 4°C. The strains were stained using methylene blue and viewed under a high power microscope (100× magnification). Colour, texture and other features were observed on the colonies. Biochemical tests of the selected yeast isolates were carried out by the means of fermentation of different carbon sources using the modified method of Olutiola et al. (2000). Photomicrographs of the isolates were taken with motic camera 2.0. The identities of the isolates were confirmed by comparing the characteristics with those of known taxa using the

Table 1. Cultural characteristics of yeast isolates.

Isolate code	EAM 2	EAM 3	EAM 5	EAM 6
Name of organism	*Kluyveromyces marxianus*	*Pichia caribbica*	*Candida tropicalis*	*Saccharomyces cerevisiae*
Pigmentation	Creamy; white	Creamy; white	Creamy	White; Creamy
Colony morphology	Raised,smooth clustered	Flat, ovoid	Flat, smooth	Oblong/Eclipse
Cell length (µm)	5.0 to 10.5	1.2 to 10.8	1.5 to 2.0	2. 0 to 8.0
Cell breadth (µm)	2.0 to 5.0	1.5 to 3.5	5.0 to 6.0	0.5 to 3.0

schemes of Rhode and Hartmann (1980) and Ellis et al. (2007). Isolates were genetically identified by growing them on MEA slants in 5.0 ml McCartney bottles and sent to the Centre for Agriculture and Bioscience International (CABI) identification service, Royal Botanical Garden Kew, England. These isolates were sequenced according to CABI standard protocols. Procedure– terms and conditions was applied (Centre for Agriculture and Bioscience International, Royal Botanical Garden Kew, England).

Determination of enzyme synthesizing ability

Urea hydrolysis test was done using the method of Seeliger (1956). The cultures were grown on Sabouraud agar and were transferred to fresh slants before the urea test was done. Fresh cultures from the slants were transferred with a loop to the surface of Christensen urea medium consisting of the following: 0.1 g peptone; 0.1 g glucose; 0.5 g NaCl; 0.2 g KH_2PO_4; 1.5 g agar and 0.012 g phenol red per 1000 ml of distilled water. The ingredients were mixed and melted in a water bath. After adjusting the pH to 6.8, the medium was dispensed into test tubes in 4.5 ml amounts and autoclaved for 10 min at 121°C. The tubes were allowed to cool to 50°C. To every tube of the autoclaved medium, 0.5 ml of a 20% Seitz-filtered solution of urea was added aseptically. After mixing with the base, the contents of the tubes were allowed to solidify with a long slant and a deep butt. The inoculated tubes were incubated at the optimal temperature (28 to 30°C) of the organism for 72 h. Urea hydrolysis was indicated by a distinct colour change of the indicator from a deep pinkish red to an orange-yellow colour starting at the slanted part of the medium and progressing rapidly to the deep part of the butt.

RESULTS

Four yeast strains were isolated, purified and further identified from different food crops produced in Nigeria. Differential tests were applied including morphological, physiological and molecular which facilitated the opportunity for identification of the yeasts. These tests allowed information gathering for the studies objectives and the determination of the systemic status of the yeast. Distinct yeast was isolated from each of the food crop with each belonging to a separate genus which included *Candida, Kluyveromyces*, *Pichia* and *Saccharomyces* (Table 1). Only one of the isolates (EAM 5) showed imperfect state and also showed a pinkish colony appearance while the others were whitish creamy. Two isolates (EAM 5 and 6) showed pseudohyphae formation. Budding was observed in isolate EAM 6 which also had butryous colony texture. All isolates showed elliptical to round spores. A single species belong to the sub-division Deuteromycotina and the rest to the sub-division Ascomycotina.

The physiological and biochemical tests of the yeasts carried out showed all isolates to ferment glucose for their growth. *Pichia caribbica* was able to ferment xylose of the four isolates. The assimilation and fermentation of most sugars by the isolates was variable. Urea hydrolysis was weak by three of the isolates with *Candida tropicalis* showing no trace of hydrolysis after 72 h, while hydrolysis was very vigorously registered by *P. caribbica* (EAM 3). Results of physiological and biochemical tests are presented in Table 2.

FASTA sequences of the studied yeast genomic DNA

Image 1 was obtained for the isolation of DNA from the four yeast isolates. The DNA sequence data obtained from some of the isolates (EAM 2, EAM 3 and EAM 5) have been deposited in the database library of the Royal Botanical Garden Kew. The blast sequence query showed that *Kluyveromyces marxianus* (IMI 398399) and *P. caribbica* (IMI 398400) had maximum identity (100%) with the genomic DNA sequence of EAM 2 and EAM 3, respectively at both ITS. Isolate EAM 5 was 100% homologous to *C. tropicalis* 18S rRNA gene sequence. The blast sequence query showed that *Saccharomyces cerevisiae* (GU 931323.1) has the maximum identity (88%) with the genomic DNA sequence of EAM 6 at ITS 1 and ITS 4 sequence with that in the Genbank Library Database (Table 3).

DISCUSSION

In this study, four yeast species from four genera were isolated from *Cola acuminata*, *M. esculenta*, *Zea mays*, and *Sorghum bicolor*. Respectively, they include *C. tropicalis*, *K. marxianus, P. caribbica* and *S. cerevisiae.*

The yeasts were found to be fermentative in the breakdown of hexose and pentose sugars. Hitherto, several workers such as Oyeleke and Jibrin (2009), Mohd et al. (2011) have reported the activities of some of the yeast strains in bioethanol production. Species of yeast like *Candida* have not been extensively reported as fermentative yeast for industrial utilization such as the

Table 2. Phenotypic characterization of the isolates.

Biochemical characteristic	Isolate			
	EAM 5	EAM 2	EAM 3	EAM 6
	C. tropicalis	*K. marxianus*	*Pichia caribbica*	*S. cerevisiae*
Gram reaction	+	+	+	+
Sucrose	-	+	+	+
Glucose	+	+	+	+
Lactose	+	V	+	+
Galactose	+	+	-	+
Maltose	+	--	+	-
Xylose	-	-	+	-
Fructose	-	+	+	+
Dextrose	-	+	-	-
Urease	-	-	+	-

+ = Present; - = absent; V = variable.

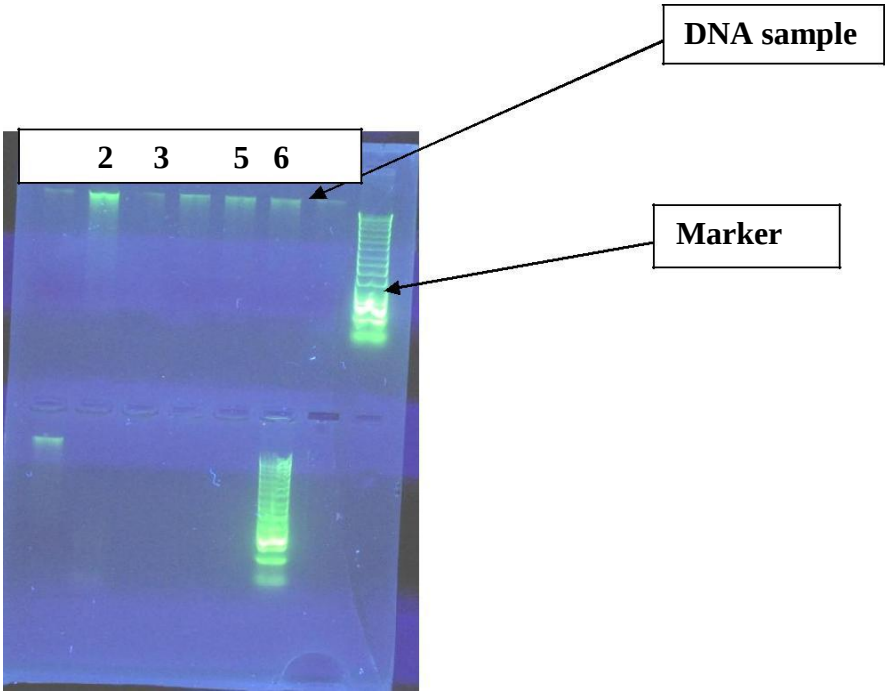

Image 1. Agarose gel electrophoresis of RAPD/PCR products of the four DNA bands (EAM 2, EAM 3, EAM 5 and EAM 6) viewed under the ultra-violet light.

production of bioethanol nor in the production of other useful organic compounds except as causal agents of human diseases. Ellis et al. (2007) reported *C. tropicalis* as the causal agent of candidiasis in man; they are opportunistic fungi which live in most human organs. However, recent reports by Kathiresan and Saravanakumar (2011) and Senthilraja et al. (2011) have shown that species of *Candida* are not just pathogens but can be useful tools for bioethanol production, as they were able to use *C. tropicalis and Candida albicans* isolated from marine environment to produce bioethanol. There had also been reports of their isolation from dairy products such as yoghurt (Rohm et al., 1992) and milk (Gadaga et al., 2000). *P. caribbica* and *C. tropicalis* were isolated from *Z. mays* and *Cola acuminate*, respectively. This is probably the first report of isolation and characterization of yeasts from these substrates that can be used in fermentation for the production of bioethanol in Nigeria. Most workers had reported the use of *S. cerevisiae* for fermentation in the production of bioethanol

Table 3. Molecular identity of yeast species isolated by the rDNA sequencing and morphological characteristics.

Isolate code	Organisms	Origin	Identification (%)	IMI (Kew, UK) identification number/accession number	rDNA[a]
EAM 2	*Kluyveromyces marxianus*	Cassava	100	IMI 398399	ITS1/ITS4
EAM 3	*Pichia caribbica*	Maize	100	IMI 398400	ITS1/ITS4
EAM 5	*Candida tropicalis*	Kolanut	100	IMI 398401	ITS1/ITS4
EAM 6	*Saccharomyces cerevisiae*	Guinea corn	88	GU931323.1	ITS1/ITS4

[a] Region of the rDNA gene used for identification.

(Abouzied and Reddy, 1986; Adesanya et al., 2008; Oyeleke and Jibrin, 2009). This report therefore gives an array of prospective fermentative species of yeast from locally available substrates which can be of industrial benefits. The organisms were able to degrade the carbon sources because they contain the enzymes necessary for the conversion of sugars to other products. Organism like *K. marxianus* was also able to ferment galactose which is an indication of the presence of *β*-galactosidase, while Rajoka et al. (2003) had earlier extracted the enzyme from *K. marxianus.* Contrary to literature reports that species of *Saccharomyces* cannot ferment lactose as they lack the enzyme lactase, the *S. cerevisiae* strain (from guinea corn) isolated in the course of this study was able to ferment the lactose used in the fermentation test. This strain could be used for the purpose of fermentation to produce alcohol and other derivatives. In yeast (fungi) taxonomy, conventional methods such as physiological and morphological analysis are not enough to adequately identify yeast especially with the emergence of new strains. Molecular identification is known to provide a more objective separation of genera and species than phenotypic analysis. DNA sequence analyses was achieved through DNA extraction using CTAB procedure, amplification of regions of rDNA/Internal Transcribed Spacer sequence (ITS) and purification of the PCR products. The amplified region was done using ITS1 and ITS4 which are recommended universal primers for fungi identifications (Trost et al., 2004). Then, the data obtained was compared with known sequence in the database of the Genbank and CABI, Royal Botanical Garden, Kew. The comparison of rDNA gene sequence is an important instrument in determining the phylogenetic and evolutionary relation-ship of many organisms. Ellepola et al. (2003) used this method to separate a species of *Candida* which had earlier been wrongly identified as *C. albicans* into the correct species of *Candida dubliniensis.* This research shows the genome rDNA/ITS sequences of the yeast samples to be more accurate and reliable in phylogenetic typing and identification than the conventional means.

Conclusion

This study has dealt with the isolation and characterization of four yeast strains using standard microbiological procedures. They showed interesting features such as extra cellular enzyme and fermentation capability which facilitate the opportunity for identification of the yeasts. The result of this study indicated that these indigenous yeasts, isolated from food crops showed good fermentation attributes, which could enhance ethanol yield that would contribute to the cost effective role in the production of bioethanol and enzymes of industrial importance; hence, increasing the varieties of yeast and decreasing its importation.

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