Yeast Strains from *Burukutu* and *Fura*, as an Alternative for Commercial Baker's Yeast

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Abstract— *Baker's yeast has been employed in the manufacturing of bread for at least 6,000 years ago. They are responsible for dough leavening and without them sugar in the dough will not be reduced and the substrate will be left unleavened. They have been identified by Scientists as Saccharomyces cerevisiae and are easily obtainable from fermenting fruits and beverages of high carbohydrate content. This work was carried out to determine the possibility of isolating baker's yeast from two local drinks, Burukutu and Fura. The drinks were prepared and allowed to ferment for 72hrs and cultured on Sabouraud Dextrose Agar (SDA) plates incorporated with chloramphenicol for 48hrs. Colonies that grow were counted and sub-cultured on Yeast Peptone Dextrose (YPD) Medium for 72hrs. Discrete colonies were sub-cultured, stored, identified and characterized. Their attributes as baker's yeast such as ethanol and stress tolerance, flocculation, hydrogen sulphide production, temperature tolerance and fermentative ability were determined. Results showed that two isolates were selected and identified as 'Isolated yeast from burukutu (IYB), Isolated yeast from fura (IYF)'. They showed similar microscopic appearance with reconstituted conventional commercial baker's yeast (CCY) such as the presence of ellipsoidal to oval cells with multipolar buds and ascospores. The multipolar buds were highest in IYB and lowest in CCY. Yeast count ranges from $3.7X10^3$ to $2.8X10^3$ colony forming unit per millilitre (cfu/ml). All the isolates were able to tolerate different concentrations of ethanol and temperature regimes at varying intensities. None of the isolates produced hydrogen sulphide but show intense to moderate response to stress, flocculation and fermentative ability. Local beverages (Burukutu and Fura) are therefore recommended to be good sources of baker's yeast which can compare favourably with the conventional commercial baker's yeast.*

Keywords— Burukutu, Fura, Saccharomyces cerevisiae, Baker's yeast.

I. INTRODUCTION

Baker's yeast had been and is still an inevitable component of raw materials used by bakers all over the world. They ferment, leaven or increase the volume of dough mixed for bread and other confectionary products through gas (CO_2) incorporation. They do not only increase the volume of the dough through carbohydrate utilization and gas incorporation but also help in creating the desired flavour and texture in the dough (Fleury *et al.*, 2002; Umeh *et al.*, 2019; Sergei, 2020; Casas-Godoy *et al*., 2021). During fermentation process in the dough, large quantity of CO_2 is produced. Baker's yeast had been identified by researchers as *Saccharomyces cerevisiae* and is the most commonly used species of *Saccharomyces* in bread baking. It has been employed as baker's yeast in manufacturing bread for at least 6,000 years ago (Kevin, 2005; Sergei, 2020; Casas-Godoy *et al*., 2021).

In Nigeria and some countries, Baker's yeast is only obtained by importation from Europe or America due to the delicate means of preservation. They are mainly used as dried, preserved as powders and are delicate to handle. Researchers had deduced that *Saccharomyces cerevisiae* does not inhabit any other environment except nature. They can be isolated from vinery environment as wild or domesticated species (Martin *et al.,* 1993; Mortimer, 2000; Umeh *et al.,* 2019). Kurtzman and Fell (1998) reported that fruits, vegetables, drinks and agricultural products are among the important micro habitats for wild yeasts.

Yeast strains are known to inhabit foods and beverages (Graham, 2007). They play an important role in the fermentation process. *Saccharomyces cerevisiae* is employed in the production of wine, beer, bread and alcoholic beverages (Mortimer, 2000; Sergei, 2020; Grijalva-Vallejos *et al*., 2020). Alcoholic drinks had been found to be good sources of *Saccharomyces cerevisiae* (Kurtzman and Fell, 1998; Graham, 2007; Umeh *et al.,* 2015; Agwuna *et al.*, 2019)

Alcoholic beverages consumed in some states in Nigeria were produced locally and can be sources of domesticated yeasts like *Saccharomyces cerevisiae.* Such beverages include *Burukutu* (local sorghum beer) and *Fura* (Millet meal). These drinks are alwaya available at all seasons of the year. Their raw materials and methods of preparation are cheap and easily available.

Baking industry is very costly in Nigeria, due to the high cost of imported bakers' yeast from developed countries, a process that drains its foreign reserve (Yabaya and Jatau, 2009, Umeh *et al.*, 2019).The scarcity of bakers' yeast has resulted in poor production of bread by bakers and making consumption of good quality bread almost beyond reach to low income earners (Yabaya and Jatau, 2009). Sometimes the imported yeasts when reconstituted fail to posses the attributes of the required species thereby rendering the production invaluable.

There is great need to look into the potentials of local beverages as sources of the yeasts that can possess the characteristics of baker's yeast to be used in our baking and confectionary industries.

II. MATERIALS AND METHODS

2.1 Sample collection

"*Burukutu*" (local sorghum beer) was obtained from a local seller at Aroma Junction, Awka with a sterile plastic container and immediately transported to the laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka.

Millet meal (*Fura*) and Commercial bakers' yeast were purchased from Eke Awka market in a sterile plastic container and cellophane bag respectively and also transported to the laboratory.

Culture media, chemicals and reagents were purchased from Head Bridge Drug Market in Onitsha, Anambra State. All the chemicals and reagents are of analytical grade and unadulterated.

2.2 Yeast Isolation and Identification

The method of Chiranjeev *et al.,* (2013) as modified and used by Umeh *et al.,* (2019) was adopted for the isolation and identification of yeasts from the *burukutu* and *fura* samples. This was done by pour plating of serially diluted samples of the *burukutu* and *fura* differently on separate plates of Sabouraud dextrose agar (SDA) incorporated with chloramphenicol. The plates were incubated at room temperature ($27\pm2^{O}C$) for 48hrs and the developed colonies counted. The different developed colonies were sub-cultured on fresh Potato Dextrose agar (PDA) plates, incubated at same condition for 72hrs to get pure cultures of the yeast strains. The isolated yeast strains were characterized and preliminarily identified using colony morphology, cellular characteristics, ascospore formation, vegetative reproduction and sugar utilization. The isolates were identified further preliminarily using the Fungal Atlas by comparing them with known taxa. They were then stored in a slant and preserved in the refrigerator at $4^{O}C$ for further genomics and gene sequencing identification using and usage.

2.3 Examination of the Isolates for Attributes of Baker's Yeast

The methods of Chi and Ameborg, (2000) as used by Umeh *et al.,* (2019) were used to check the isolates for their attributes as baker's yeast. The following attributes were determined:

2.3.1 Ethanol Tolerance test

The ability of the isolated strains to grow and survive in high concentrations of ethanol was tested by growing them in Yeast Peptone Glucose (YPG) broth containing three different concentrations of ethanol. One loop full of each isolate was inoculated into freshly prepared YPG broth, containing ethanol concentrations of 10, 15 and 20% (v/v) respectively and incubated for 72hrs and observed for survival and multiplication.

2.3.2 Stress Tolerance

The ability of the isolates to survive at different stress conditions was done for consecutive 15 days' inoculation and incubation on different media. This was conducted by first growing the isolates on normal YPG for 3days, then transferring them on YPG

medium containing 8% (v/v) ethanol and 20% (w/v) glucose for another 3 days and finally inoculating them on YPG medium with 8% (v/v) ethanol and 20% ((w/v) sucrose for the last 3 days, all incubated at the same condition of temperature.

2.3.3 Flocculation Test

A loop full of each of the isolates was inoculated in a test tube containing 10 ml fresh YPG broth and incubated at 30^{O}C for 72hrs. The tubes were observed after 72hrs and agitated to observe flocculate formation.

2.3.4 Hydrogen sulphide production

The ability of the isolated yeasts to produce hydrogen sulphide was checked by growing them on Lead acetate medium which is composed of 40 g/l Glucose, 5 g/l Yeast extract, 3 g/l Peptone, 0.2 g/l Ammonium sulphate, 1 g/l Lead acetate and 20 g/l Agar. The set up was incubated for 30 ^{O}C for 10 days and the media tested for the presence of hydrogen sulphide.

2.3.5 Temperature Tolerance Test

The ability of the isolates to grow at varying degrees of temperatures was checked by culturing them on YPG medium and incubating them at three different temperature regimes of 30 ^{O}C, 35 ^{O}C and 45 ^{O}C. The isolates were streaked on the medium and incubated for 72hrs.

2.3.6 Fermentative ability

The ability of the isolates to ferment different carbon sources was checked in this test. Solutions of 10 ml 6 % (w/v) each of glucose, sucrose, fructose, galactose, raffinose, lactose and maltose were autoclaved differently with inverted Durham tubes and cooled. The isolates were each grown on YPG at 30 ^{O}C for 72hrs. They were then inoculated on fresh Yeast fermentation broth (YFB) made of 7.5 g/l Peptone, 4.5 g/l Yeast extract, and 1 ml of 1.6% (w/v) Bromothymol blue as indicator. Equal quantities of the YFB were aseptically added to each of the tubes containing the different carbon sources and 2 loop fill of each isolate inoculated. They were incubated at 30 ^{O}C for 72hrs and the tubes examined for turbidity, acid and gas formation.

III. RESULTS

The isolates were identified and designated as Isolated yeast from *burukutu* (IYB), and Isolated yeast from *fura* (IYF) while the Conventional commercial yeast was designated as CCY. They show the same type of microscopic appearance of presence of ellipsoidal to oval cells with multipolar buds and ascospores. The multipolar buds were highest in IYB and lowest in CCY. Yeast count ranges from 3.7X10^{3} to 2.9X10^{3} colony forming unit per millilitre (cfu/ml) as shown in Table1.

TABLE 1
COLONY MORPHOLOGY OF THE ISOLATES ON YPD AND YEAST COUNTS ON SDA

Isolates	Creamy	fluffy	Smooth	Rough	Yeast counts X10^{3}cfu/ml
IYB	-	+	-	+	3.7
IYF	-	+	+	+	2.9
CCY	-	+	-	+	2.8

Key: - (Negative reaction), + (Positive reaction), cfu/ml (Colony forming unit per millilitre)

The isolates were able to tolerate different concentrations of ethanol and survive different temperatures regimes at varying intensities as presented in Table 2.

TABLE 2
ETHANOL AND TEMPERATURE TOLERANCE ABILITY OF THE ISOLATED STRAINS

Isolates	Ethanol concentrations (%)			Varying temperatures (ºC)		
	10	15	20	30	35	40
IYB	+++	+++	+++	+++	++	++
IYF	+++	+++	++	+++	++	+
CCY	+++	++	++	+++	+	+

Key: +++ (Intensive growth) ++ (Moderate growth) + (Low growth)

Table 3 showed the results of stress exclusion test, flocculation, hydrogen sulphide production and fermentative abilities of the isolates.

TABLE 3
STRESS TOLERANCE, FLOCCULATION ABILITY, HYDROGEN SULPHIDE PRODUCTION AND FERMENTATIVE ABILITY OF THE ISOLATES.

Isolates	Stress Tolerance	Flocculation	H_2S production	Fermentative ability
IYB	+++	+++	-	+++
IYF	+++	++	-	+++
CCY	+++	++	-	++

Key: +++ (Intensive response) ++ (Moderate response) + (Low response)

The isolated yeasts and the conventional baker's yeast were able to ferment glucose, sucrose, fructose, galactose, and maltose with extensive to moderate gas production. None of the yeasts were able to ferment raffinose and lactose (Table 4).

TABLE 4
SUGAR FERMENTATION ABILITIES OF THE THREE ISOLATES

Isolates	Glucose	Sucrose	Fructose	Raffinose	Galactose	Lactose	Maltose
IYB	+	+	+	-	+	-	+
IYF	+	+	+	-	+	-	+
CCY	+	+	+	-	+	-	+

Key: + (Able to ferment) - (Unable to ferment)

IV. DISCUSSION

Baker's yeast had been identified by researchers as *Saccharomyces cerevisiae* (Benitez *et al.,* 1996; Thais *et al.,* 2006; Umeh *et al.,* 2019). The primary role of this yeast in baking is in dough development where it ferments the carbon source in the dough with simultaneous production of carbon dioxide thereby raising/leavening the dough. Without baker's yeast the fermentation and leavening of the dough would be impossible. Importation of baker's yeast had rendered some baking and confectionary industries weak in production due to its high cost. Most times, due to long storage, mutation of the yeasts may occur in transit rendering the yeast unsuitable for use and great loss to the producer. It had also led to high cost of bread and other backed products as the cost as well as the cost of baking flour are increasing day after day (Umeh *et al.,* 2017; Agwuna *et al.,* 2019).

Two Nigerian fermenting local beverages (*burukutu* and *fura*) were assessed for the presence of the baker's yeast. The two fermenting drinks when cultured on Sabouraud Dextrose Agar, showed the presence of fungal colonies after 48hrs. From the discrete colonies, the yeasts were isolated and selected based on their morphology, microscopic appearances and biochemical characteristics and enriched as baker's yeast (Table 1). Their colony appearances of Creamy, Fluffy, Smooth and rough/regular were attributes of known baker's yeast. This was in agreement with the report of Cavalieri *et al.,* (2001), Kuthan *et al.,* (2003), Kevin (2005) and Umeh *et al.,* (2019). The isolated yeast cells showed profuse budding capacity which is also a good feature of industrial baker's yeasts (Umeh and Okafor 2017). Colony growth on Sabouraud dextrose agar and yeast Peptone Agar were profuse and colony count much more than the reconstituted commercial yeast (Table 1). The conventional commercial yeast may show the same count range if higher dilution was cultured.

Table 2 showed the result of ethanol and temperature tolerance of the yeasts. All the three yeasts were able to withstand ethanol concentrations of 10% and grow profusely, the two isolated in the research grew profusely at 15% ethanol while the commercial baker's yeast showed moderate growth at 15% ethanol concentration. This is in line with the findings of Iraj *et al.,* (2002), Irena *et al.,* (2005) and Chilaka *et al.,* (2010). They also grow well at temperatures of 30 ^{O}C showing profuse growth, IYB and IYF grew moderately at 35 ^{O}C and scanty at 45 ^{O}C while CCY grew scantly at these temperatures (Table 2). This report supported the findings of Chiranjeev *et al., (*2013) which said that yeast isolated from local/natural sources can survive high temperature regimes.

Both the yeasts isolated from the research and the commercial baker's yeast did not produce hydrogen sulphide on growth on yeast peptone glucose medium (Table 3). This is a good attribute of good baker's yeast as postulated by Irena *et al.,* (2005), Chilaka *et al.,* (2010) and Umeh and Okafor, (2016). The studied yeast strains were able to withstand stress on culturing them on media of different harsh conditions. IYB and IYF showed intensive growth under stress for 15 days while CCY show moderate growth response. IYB developed better flocculation in broth than IYF and CCY which developed scanty flocculates (Table 3). Baker's yeast tolerance to stress and ability to flocculate had been found to be good characteristics of baker's yeast as found and confirmed by Irena *et al.,* (2005) and Chilaka *et al.,* (2010). These two characteristics of baker's yeast help them to survive in different ingredients in the dough mixture without denaturing and at the same time do the function dough leavening (Umeh and Okafor, 2016).

The isolated yeasts in this research, Isolated *burukutu* and Isolated *fura* yeasts (IBY and IFY respectively) were able to ferment the tested carbohydrate sources (glucose, fructose, sucrose, galactose and maltose) but did not ferment raffinose and lactose. This is in line with the findings of Thais *et al.,*(2006) and Umeh *et al.,* (2019). Tarek (2001) reported that the *Saccharomyces cerevisiae* strains that do not ferment lactose lack the enzyme lactase or β-galactosidase system. The studied yeast species from this fermented the carbon sources with the production of gas. The gas produced was carbon dioxide which represents the carbon dioxide released during fermentation and leavening processes in bread making and this is paramount in bakery (Thias *et al.,* 2006).

V. CONCLUSION

The two local drinks studied showed good sources of baker's yeasts which competes and is found to be more potent than the conventional baker's yeast. These isolated yeasts can be potentially employed in baking and confectionary industries to reduce the cost on importation of the conventional baker's yeasts.

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