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Screening of Fermentative Potency of Yeast Isolates from Indigenous Sources for Dough Leavening

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Abstract: Baker's yeast is one of the most essential imported ingredients in baking industries/bakery in Nigeria. Yeast as a common organism in our environment, there is a greater possibility to recover and identify baker's yeast from various indigenous sources. This study was focused on isolating, identifying and assessing the dough fermenting abilities of yeasts from various indigenous sources which can potentially be employed as a leavening agent. Differential test were applied including cultural, morphological and biochemical characteristics (using API20C AUX Kit (BioMeriux), which facilitated the identification of the yeasts to specie level. The isolates were subjected to baking potency test; ethanol tolerance test, hydrogen sulphide (H_2S) production test, temperature tolerance test, flocculation test (effect of shaking) and leavening action on bread dough. Thirteen (13) yeasts were isolated from sweet orange (*Citrus sinensis*), pineapple (*Ananas comosus*) and palm wine. These isolates belong to the genera of *Candida, Rhodotorula, Kodamaea and Cryptococcus.* Four yeast Species namely; *Candida colliculosa, Candida krusei, Rhodotorula mucilagnosa and Rhodotorula minuta* were used to ferment wheat flour dough in order to determine their individual fermentative abilities where *Rhodotorula minuta* and *Rhodotorula mucilagnosa* showed better performance compared to commercial yeast. Thus indicates that the local fruit could be a potential source of indigenous yeast species for leavening agent in bread making.

Keywords: Fermentative Potency, Leavening Ability, Yeast, *Citrus Sinensis and Ananas Comosus*

1. Introduction

Leavening agents either chemical or biological are important in raising flour dough. Biological leavening agents are microorganisms that have the ability to produce carbon dioxide from the utilization of sugar [1]. Yeast plays an important role in various fermentation processes including baking and brewing. In brewing, the alcohol released by the fungus during fermentation is important while carbon dioxide is of utmost need for rising of flour dough, maturation and development of fermentation flavour [2] [3].

Yeasts have been isolated from various fermenting sources for dough leavening. Such include yeasts isolated from Nigerian palm wine [4] [5] and pineapple [6]. The useful physiological properties of yeasts have led to their use in the field of industrial microbiology. Fermentation of sugars by yeast is the oldest application in the making of bread, beer and wine.

Orange (*Citrus sinensis*) juice is an acidic beverage (pH 3 to 4) with high sugar content (~15° Brix). Under these conditions, acidolactic bacteria, moulds, and yeasts comprise the typical microbiota present in citrus juices [7]. According to [8] typical yeast species associated with citrus juices are *Candida parapsilosis, Candida stellata, Saccharomyces cerevisiae, Torulaspora delbrueckii,* and *Zygosaccharomyces rouxii.* [5] Revealed that yeasts occur generally in sugary substances like palm wine, maple syrup, flower parts and cane juice. Accordingly, [5] asserted that palm wine contains yeasts which belong largely to genus *Saccharomyces*. It also contains *Kloeckera, Pichia* and *Candida.*

Pineapple contains good sugar proportion which makes it suitable for yeast growth [9]. Previous study on the microbiology of pineapple showed that several bacteria and yeasts were isolated and identified as the genera *Saccharomyces, Rhodotorula, Candida* [10].

This study focused on isolating, identifying and assessing the dough fermenting ability of yeasts from various indigenous sources which can be employed as a leavening agent.

2. Materials and Methods

2.1. Isolation of Yeasts from Different Sources

Fully ripe orange and pineapple fruits were obtained from the Institute of Agricultural Research, Ahmadu Bello University, Zaria. The fruits were washed, cut, squeezed and the juice was collected into a sterile air-tight container and allowed to ferment naturally for 2-4 days. Freshly tapped palm wine was allowed to ferment for 24 hours. 1ml of the serially diluted samples was plated on Potato dextrose agar (PDA) by using pour plate method [11] and incubated at 30°C for 48-72 hours. After growth of colonies, each type of colony was sub cultured on fresh PDA by streaking. Pure isolates were preserved on agar slants and stored at 4°C until needed. Each colony was confirmed to be yeasts by examining them under a light microscope at 100x magnification after staining with methylene blue dye [11].

2.2. Identification of Yeast Isolates

The yeast isolates were characterized based on their cultural and morphological characteristics as described by [12]. The cultural properties of the yeast isolates were determined by observing their distinct colonies on PDA. Colony shape, size, colour, elevation were the cultural characteristics considered, morphological characteristics including; cell shape and bud. Biochemical characterization of the yeasts was done using the API20C AUX KIT (BioMeriux) according to the method described by [13].

2.3. Ethanol Tolerance Test of Yeast Isolates

The ability of the yeast species to grow in higher ethanol concentrations medium were tested by growing them in Yeast peptone glucose (YPG) broth containing 3 different concentrations of ethanol, 10%, 13% and 15% (v/v), respectively and incubated at 30°C for 72 hours [11].

2.4. Temperature Tolerance Test of Yeast Isolates

The ability of the yeast to grow at higher temperatures was verified by plating the yeast isolates onto Yeast Peptone Glucose (YPG) medium and was incubated at 5 different temperatures (25, 30, 37, 45 and 50°C) for 72 hours. After incubation, yeast isolates that survived at high temperature indicates that they may be used in bread making [11].

2.5. Hydrogen Sulphide (H_2S) Production Test of Yeast Isolates

The ability of the yeast to produce hydrogen sulphide (H_2S) was examined by growing the yeast isolates on Kligler Iron Agar (KIA) and incubated at 30°C for 10 days [14].

2.6. Flocculation Test (Effect of Agitation) of Yeast Isolates

Yeast isolates were inoculated in 10ml of Yeast Peptone Glucose (YPG) broth separately and incubated at 140 rpm and 30°C in shaking incubator for 3 days to observe the flocculation formed. After incubation, the culture broth was centrifuged at 5000 rpm for 10 minutes, the supernatant was decanted. The pellets were weighed to determine biomass production in shake culture [11].

2.7. Leavening Action of Yeast Isolates

Dough containing wheat flour (200g), sugar (25g), salt (5g) and water (100ml) was prepared and inoculated with yeast isolates using 0.5 McFraland standards. Baker's yeast was used as positive control to ferment the dough. Another set of dough without yeast was prepared as a negative control sample. The prepared dough samples were inserted into a 1000 ml beaker aseptically and left to ferment at room temperature (37±2°C) for 6 hours interval up to 36 hours. Baking potency was determined by measuring the volume increase, in the beaker [15].

3. Results and Discussion

A total of 13 yeast isolates were obtained based on their morphological and physiological characteristics and were identified to specie level using API20C AUX KIT (biomeriux) from the samples of sweet orange (*Citrus sinensis*), pineapple (*Ananas comosus*) and palm wine. The result of the cultural characteristics showed that the colonies of the yeast isolates were white, creamy, circular, triangular, smooth, raised and flat (Table 1). Similar findings were also observed by [14] who reported that typical yeast colonies were creamy and regular colony shape. Microscopically, the vegetative cells are oval, circular in clusters and chains with budding (Table 2). Yeast capable of growing on solid surfaces tends to form colonies with distinctive morphology; this is because individual species often form colonies of characteristics size and appearance as reported by [17]. This statement agrees with this study because all the yeast species isolated were similar in colonial appearance and vegetative morphology that shared the same oval, spherical shape and budding as a means of asexual reproduction.

Thirteen (13) of the isolates were identified to be representatives of the following yeast species: *Candida colliculosa, Rhodotorula minuta, Candida krusei, Candida magnolia, Rhodotorula mucilagnos, Cadida utilis, Cryptococcus leurentii and Kodamaea ohmeri.* The remaining two (2) isolates could not be identified. This could be associated with the absence of their biochemical properties in the reference library used for the identification.

All yeast isolates assimilated glucose except *Candida magnolia*; this implies that it does not possess enzymes responsible for the utilization of this sugar. All the yeast species did not assimilate adonitol, xylitol and methyl- D Glucosid except *Cryptococcus leurentii.* The inability of other yeast isolates to metabolise adonitol, xylitol and methyl- D Glucosid indicates that these species lack the reductase and dehydrogenase genes responsible for the fermentation of these sugars [18]. None of the yeast species assimilated galactose and lactose. According to [18], galactose is a non-conventional nutrient for yeasts, which however can be used as sole carbon source when glucose is absent from the medium. Thus the ability of yeast cells to assimilate galactose indicates

the expression of GAL genes. This infers that yeast isolates that could not assimilate galactose do not possess GAL genes.

From table 3, it can be seen that *Cryptococcus leurentii* was found to be the most versatile yeast isolate with the ability to ferment most of the sugars. This indicates that it has enzymes responsible for fermentation of most of the sugars.

Table 4, revealed that all the yeast species were able to grow in a medium containing 10% (v/v) of ethanol, at 13% (v/v) of ethanol concentration, only yeast specie PW6 (isolated from palm wine) was not able to grow. Yeast species CS2, CS7, CS8 (isolated from sweet orange) and AC5 (isolated from pineapple) showed slow growth in 15% (v/v) of ethanol, while other yeasts failed to grow at that concentration. The results are in line with the findings of [19] who observed that yeast species were able to grow in a medium containing 10% and 13% (v/v) of ethanol concentration while only few yeast species could grow in 15% (v/v) ethanol. High concentration of alcohol is reported to be toxic to the yeast by inhibiting the cells growth due to the destruction of the cell membrane [20]. This could have accounted for the slow growth of most of the isolates at 15% concentration as recorded in this research. A suitable concentration of alcohol is needed in bread making in order to achieve the preferred flavour [20].

All the yeast species could tolerate a temperature up to 45^{o}C except PW6 (Table 4). The result in this research contradicts the findings of [21], who observed that yeast isolates could only tolerate a temperature up to 37°C. The ability of yeast to tolerate high temperature suggests that the isolates can withstand excess heat associated with fermentation process and therefore can be used to accomplish fermentation at wide range of temperature condition. They may also be used in bread making to speed up the proofing process, increase carbon dioxide production and formation of flavour and aroma may be enhanced.

Nine (9) yeast species (AC2, AC4, AC5, CS7, CS8, PW1, PW2, PW4 and PW6) produced H_2S. However species AC1, AC3, CS2 and PW7 did not (Table 5). Hydrogen sulphide (H_2S) is an undesirable compound associated with an off-flavour and unpleasant taste that must be absent in processed foods [22]. Yeasts that showed high production of H_2S are undesirable for bread making because it confer flavour and taste that compromise the quality of the bread [22].

Therefore, the yeast species that did not produce H_2S could be recommended as the best candidate isolates in bread making.

Figure 1, revealed the effect of shaking condition on biomass production by the yeast isolates where all the isolates showed higher biomass production in shaking condition than non-shaking condition. This result is in conformity with the findings of [23] who observed that yeast species show higher biomass production in shaking condition than non-shaking condition. Shaking or agitation creates aerobic condition in the broth, it enhance the biomass production by the isolates [24]. According to [25] [26], yeast cells which have ability to flocculate cause by cell adhesion process is an interesting characteristic in bread making and in brewing industry. This phenomenon has an economic effect on the production of yeast biomass because it can reduce the energy cost of biomass centrifuging [27]. In addition, flocculation properties of yeast species ensure a high cell density and large volume of harvested cells and also able to rise the ethanol productivity during fermentation process [16].

From the findings of this research, it could be deduced that shaking/ agitation favours the biomass production of the yeast isolates.

The leavening properties of dough fermented with the various yeast isolates from local fruits are showed in (Figure 2). The selected yeasts isolates exhibited excellent leavening capacity where yeast specie CS2 had the highest leavening ability while dough fermented without yeast (negative control) had the lowest leavening activity rating. The high leavening performance of CS2 indicates that the yeast was the best biological wheat dough leavener obtained in this study. The result indicates that the fermentative potency of the yeast isolates is even better than the commercial yeast tested. This result is in line with the findings of [21] [19] who observed that yeast species isolated from local fruits and plants part showed better fermentative ability to leaven bread dough than commercial yeast. Therefore, the yeast isolate could be considered as an excellent alternative to commercial baker's yeast to ferment bread dough.

Table 1. *Cultural Characteristics of Yeast Isolates after 48 hours of Incubation.*

Isolates	Shape	Elevation	Colour
Pineapple			
AC1	Circular	Slightly raised, smooth	Orange
AC2	Circular	Slightly raised, smooth	Orange
AC3	Circular	Flat, spread	Creamy
AC4	Circular	Flat, spread	Creamy
AC5	Circular	Flat, spread	Creamy
Sweet orange			
CS2	Circular	Raised, smooth in clusters	Creamy white
CS7	Circular	Raised, smooth	White
CS8	Circular	Flat, smooth	White
Palm wine			
PW1	Circular	Raised, smooth	Creamy
PW2	Circular	Raised, smooth	Creamy
PW4	Triangular	Raised, smooth	Creamy
PW6	Circular	Raised, smooth	Creamy
PW7	Circular	Raised, smooth in clusters	White

Table 2. *Morphological Characteristics of Yeast Isolates.*

Isolates	Cell shape	Bud
AC1	Oval, in clusters	Present
AC2	Oval, in clusters	Present
AC3	Oval, in clusters	Present
AC4	Circular, in clusters	Absent
AC5	Oval, in clusters	Present
CS2	Oval, in clusters	Present
CS7	Oval, in clusters	Present
CS8	Oval, in clusters	Present
PW1	Oval, in chains	Present
PW2	Oval, in clusters	Present
PW4	Oval, in clusters	Present
PW6	Circular, in clusters	Absent
PW7	Oval, in clusters	Present

Key: AC (Pineapple isolates), CS (Sweet orange) and PW (Palm wine).

Table 3. *Characterization of Yeast Isolates using API20C AUX Kit (BioMeriux).*

Isolates	GLU	GLY	2KG	ARA	XYL	ADO	XLT	GAL	INO	Identity
AC1	+	+	+	-	-	-	-	-	-	*Candida colliculosa*
AC2	+	+	-	-	+	-	-	-	-	*Rhodotorula minuta*
AC3	+	-	-	-	-	-	-	-	-	*Candida krusei*
AC4	+	-	-	-	-	-	-	-	-	*Candida magnolia*
AC5	-	+	+	+	-	-	-	-	-	*Candida magnolia*
CS2	+	+	-	-	+	-	-	-	-	*Rhodotorula mucilagnosa*
CS7	+	+	-	-	-	-	-	-	-	*Kodamaea ohmeri*
CS8	+	+	+	-	-	-	-	-	-	*Rhodotorula minuta*
PW1	+	-	-	+	-	-	-	-	-	*Cryptococcus terreus*
PW2	+	+	-	-	+	-	-	-	-	*Candida utilis*
PW4	+	+	+	+	+	-	-	-	-	*Rhodotorula minuta*
PW6	+	+	+	+	+	+	+	-	+	*Cryptococcus leurentii*
PW7	+	+	+	-	-	-	-	-	-	*Rhodotorula minuta*

Table 3. *Continue.*

Isolates	SOR	MDG	NAG	CEL	LAC	MAL	SAC	TRE	MLZ	RAF	Identity
AC1	-	-	-	-	-	-	-	-	-	-	*Candida colliculosa*
AC2	+	-	-	-	-	+	-	-	-	+	*Rhodotorula minuta*
AC3	-	-	+	-	-	-	-	-	-	-	*Candida krusei*
AC4	-	-	-	-	-	-	+	-	-	-	*Candida magnolia*
AC5	-	-	-	-	-	+	+	+	+	+	*Candida magnolia*
CS2	-	-	+	+	-	+	-	+	-	-	*Rhodotorula mucilagnosa*
CS7	-	-	+	-	-	-	+	+	-	-	*Kodamaea ohmeri*
CS8	-	-	+	-	-	-	-	-	-	-	*Rhodotorula minuta*
PW1	-	-	+	+	-	+	+	+	-	+	*Cryptococcus terreus*
PW2	-	-	+	+	-	+	+	+	-	+	*Candida utilis*
PW4	-	-	-	+	-	+	+	-	-	+	*Rhodotorula minuta*
PW6	+	+	-	+	-	+	+	-	-	+	*Cryptococcus leurentii*
PW7	-	-	+	+	-	+	+	-	+	+	*Rhodotorula minuta*

Key: AC (Pineapple isolates), CS (Sweet orange), PW (Palm wine), GLU- Glucose, GLY- Glycerol, 2KG- 2-Ketoglutanate, ARA-Arabinose, XYL- D- Xylose, ADO- Adonitol, XLT- Xylitol, GAL- Galactose, INO- Inositol, SOR- Sorbitol, MDG- A Methyl- D Glucosid, NAG- N- Acetyl D- Glucosamine, CEL- Celliobiose, LAC- Lactose, MAL- Maltose, SAC- Saccharose, TRE- Trehalose, MLZ- Melezitose, RAF- Raffinose, NI- Not identified.

Table 4. *Effect of Ethanol Concentration and Temperature on Yeast Species.*

Yeast Species	Ethanol Concentration (v/v)			Temperature (°C)				
	10%	13%	15%	25	30	37	45	50
AC1	+	+	-	+	+	+	+	-
AC2	+	+	-	+	+	+	+	-
AC3	+	+	-	+	+	+	+	-
AC4	+	+	-	+	+	+	+	-
AC5	+	+	+	+	+	+	+	-
CS2	+	+	+	+	+	+	+	-
CS7	+	+	+	+	+	+	+	-
CS8	+	+	+	+	+	+	+	-
PW1	+	+	-	+	+	+	+	-
PW2	+	+	-	+	+	+	+	-
PW4	+	+	-	+	+	+	+	-
PW6	+	-	-	+	+	+	-	-
PW7	+	+	-	+	+	+	+	-

Key: + (Presence of growth), - (No growth).

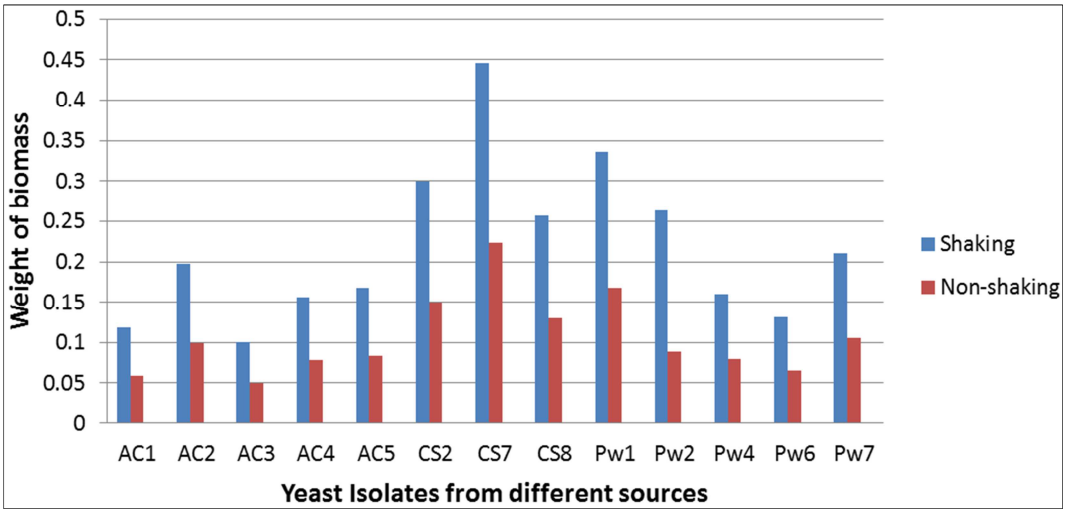

Figure 1. *Effect of Shaking Condition on Biomass Production by the Yeast Isolates.*

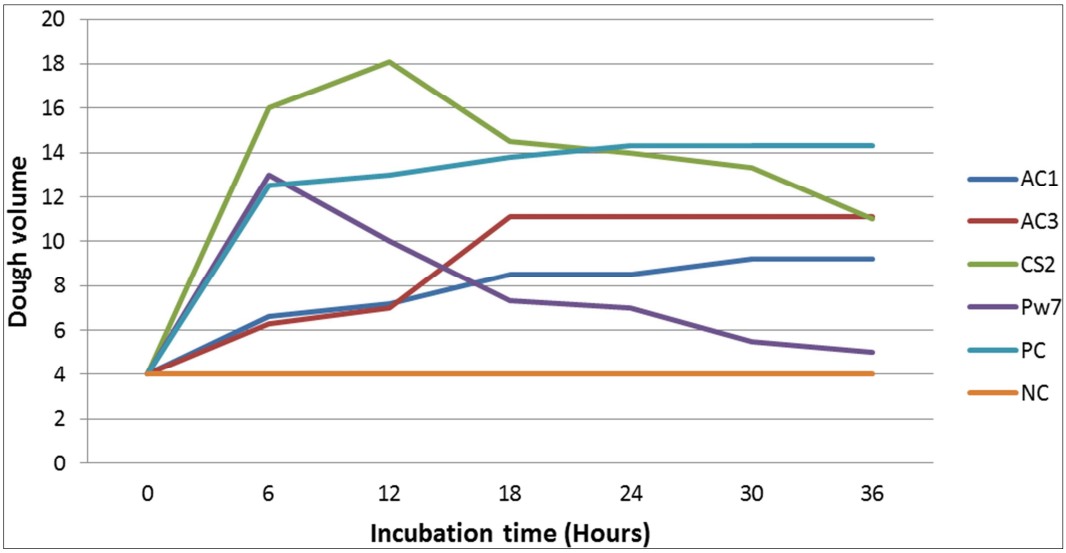

Figure 2. *Leavening Action of Yeast Isolates, in relation to Incubation Time.*

Table 5. *Hydrogen Sulphide (H_2S) Gas Production by Yeast Isolates.*

Blackening by Hydrogen Sulphide (H_2S) on KIA (Kligler Iron Agar)			
Isolates	**KIA**	**Bad odour**	**Remarks**
AC1	-	-	++
AC2	+	+	D
AC3	-	-	++
AC4	+	+	D
AC5	+	+	D
CS2	-	-	++
CS7	+	+	D
CS8	+	+	D
PW1	+	+	D
PW2	+	+	D
PW4	+	+	D
PW6	+	+	D
PW7	-	-	++

Key: - (Absent), + (Present), ++ (Recommended for further test), d (Discard).

4. Conclusion

In conclusion, local fruits are excellent habitat where yeasts with potentials for industrial uses can be isolated, particularly yeasts with dough fermenting ability as observed in this research. In all the attributes considered, the yeast, *Rhodotorula mucilagnosa* (CS2) had the overall best performance compared to commercial yeast. Other isolates that showed good performance are *Rhodotorula minuta* (PW7), *Candida colliculosa* (AC1) and *Candida krusei* (AC3). It was evidence from these findings that local fruits could be source for baker's yeast which is potentially used as dough leavening agent.

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