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Assessment of Industrial Potentials of Some Yeast Isolates from Pawpaw, Banana and Orange Fruits Grown Locally in Bauchi State

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Abstract

A study of the industrial potentials of yeast isolates from pawpaw, banana and orange fruits grown locally in Bauchi state was carried out. The parameters analyzed includes estimation of yeast potency, flocculation rate, ethanol tolerance, Protein and Vitamin content. The three species of yeast identified include Saccharomyces cerevisiae A,B,C,D. Candida tropical C,D and Saccharomyces ferméntati B,C,D. The result of the analysis showed that all strains compared favorably with the industrial yeast with respect to temperature tolerance, protein and Vitamin content. However, Saccharomyces cerevisiae A,B,C,D compared most favorably with the industrial yeast in viability, tolerance to alcohol concentration up to 20% and a high flocculation rate of 0.75m1/10minutes than Candida tropicalis C,D and Saccharomyces fermentati B,C,D. Furthermore, the temperature tolerance showed no difference with the standard at both 37℃ and 42℃ at 5% level of significance. The importance of mass production yeast strains have been stressed.

Keywords: *Assessment, Potentials, Yeast isolates, pawpaw, banana and orange fruits.*

Introduction

Yeast are fungi of the ascomycetes particularly of the group hemiascomycetidae. They belong to the order Endomycetales and family Saccharomycetae. There are many genera known under this family, but only a few have species and strains that are of use to man. They include strains that are used in food industries either as food additives or in bakeries for the rising of dough, as well as in pharmaceutical, fermentation and brewing industries. (Satish, *et al*., 2010). These strains of industrial importance mainly belong to the genus Saccharomyces . Some species under this genus include *saccharomyces cerevisiae, Saccharormyces bayanus, saccharomyces lactis, Saccharomyces carlsbergensis, Saccharomyces fermentati and Saccharomyces kluyyeri* among others. (Deak, 2017). However some pathogenic species belong to the genus Candida. Food yeast in today's market is represented by these species of the genus Saccharomyces and two species of the genus Candida. (Deak, 2017).

The use of yeast cannot be over emphasized, they are the most important raw materials of bakeries, pharmaceutical and brewing industries as well as in the production of laboratory alcohols, spirits and gins of all sort. More recently, yeast has been used in the biofuel industry and for the production of heterologous compounds, (Qureshi, *at al*., 2015). It is in view of their paramount importance that this work looks into finding out ways of assessing the industrial potentials of yeast strains from local sources as against the present imported strains used in the industry.

Furthermore, the ready availability of these local sources (fruits) will further help to minimize the cost implication on the Nation if exploited. The dependence on imported yeast does not positively correlate efforts towards backward integration less dependence on importation. Hence the possibility of using yeast isolates from banana, pawpaw and orange as an alternative to the imported strains .

MEATERIAL AND METHODS

Sample collection

The industrial yeast and other materials for Isolation (pawpaw, Banana and orange) were purchased from "Wunti markets, Bauchi state.

MEDIA PREPARATION FOR ISOLATION OF YEAST STRAIN

Yeast extract agar was used as medium for isolation, The medium was prepared according to the manufacturer instructions and was sterilized at 121^{o}C for 15 minutes. Chloramphenicol was incorporated into the agar to inhibit bacterial growths. The prepared medium was then dispensed aseptically into sterile Petri dishes and left on bench to solidify.

MEDIA FOR MAINTAINING YEAST CULTURE

The same procedure and media type for isolation was used in maintaining the isolated yeast cells. However instead of using Petri-dish, yeast extract agar slants were used. These were then stored in refrigerator at 4^0 C .

SUGAR ASSIMILATION TEST

Solution of 0.4% yeast extract and 0.7% bacteriological peptone was prepared from which 4ml each of the solution was dispensed into sterile test tube. Bromothymol blue indicator was added to each of the test tube until the solution become intense blue in color. Inverted Durham tubes were then inserted into each the tubes and the solutions sterilized at 121^{o}c for 15 minutes. The solutions of glucose, sucrose, maltose and lactose sugars were individually prepared. From each solution 2m1 was pipetted into the cooled solutions respectively and mixed well using sterile rod. These were then inoculated with the isolated yeast strains, such that the isolates were inoculated into four test tubes of the solutions containing each of the four sugar type with the yeast strains isolated from pawpaw , banana, orange and industrial yeast respectively, Piton, (2014).

ISOLATION OF YEAST CELLS

To obtain isolated discrete colonies, a 10 - fold dilution up to 10^4 was made. A volume of 0. I ml of each dilution was pipetted into the surface of yeast extract agar plate and incubated. Suspected colonies were picked and stained with carbol fuchsine. Colonies that gave a positive result from the sub culture were further identified, Amose, *et al*., (2006).

IDENTIFICATION AND CHARACTERIZATION YEAST ISOLATES

The identification of the yeast strains obtained was based on culture morphological and physiological properties of the yeast. Helm, *et al*., (2016).

Cultural characteristics

The cultural characteristics were examined by direct study of the various colonies on the agar plate. The criteria used include color, edge and level .

Morphological identification was carried out by staining with methylene blue and observed under the low power objective (x10). The criteria used was based on the shape, size of the yeast and presence of either mycelium or pseudo mycelium .

Sugar assimilation test

Each set of the four sugars was inoculated with 0.1 ml of the yeast isolates suspension and incubated at 28℃ for 4 days. Positive assimilation on reaction was indicated by the presence of color change and gas bubbles in Durham tubes.

ESTIMATION OF YEAST POTENCY

This was done according to the method of Price, *et al.,* (2009)

Viability of yeast strain

The different yeast suspension were fixed on a microscope slides and the smears were stained with methylene blue solution diluted in 1:10, 000 and covered with a coverslip. These were then examined under the microscope. Viable yeast cells appear colorless while Cozad cells appear blue.

Test for glycogen

A suspension of yeast cells was fixed on the microscope slide, covered with Lugol's iodine and observed under the microscope. Yeast cells containing polysaccharides glycogen are stained dark brown, while the yeast cells without glycogen are stained yellow.

Test for lipids

Suspensions of yeast cells were fixed on a microscope slide, stained with Sudan 111, solution, and observed under the microscope. Yeast cells containing lipids are strained red while the cells without lipids are colorless.

CHRACTERIZATION OF THE YEAST ISOLATES as described by Burns and modified by Helm, *et al*., (2016)

Flocculating

Flocculating ability of the Yeast was determined by the rate of sedimentation of washed cells in a buffer. The yeast where grown for 2 days and harvested by centrifugation at 1000rpm for 20 minutes . It was then washed twice with sterile distilled water, dried with filter papers. I.0g of washed and centrifuged yeast (wet weight) was suspended in 10ml of distilled water and 1ml of acetate buffer (pH 3. 6) was added. The suspension was shaken thoroughly in a 15ml centrifuge tube and allowed to stand for 10minites. The amount of sediment formed after 10minutes indicate whether or not the yeast was flocculent. {0.5-1.0 ml flocculent: 0.0-0.4ml non flocculent)

Ethanol tolerance

The method was based on visual assessment of growth in a test tube. To a medium containing graded concentrations (10%, 158%; 20, v/ v) of ethanol 0.5mls of a growing culture of yeast were added. The Test tubes were than incubated at room temperature and after 24 hours of incubation subsequent culture were made with a wire loop to a sterile tube with a medium. The inoculated test tubes were then incubated and subsequent examination for growth after 2 days was observed. The medium used was made up w/v 2% sucrose, 0.3% yeast extract and 0.5% peptone, 1.5% peptone.

Temperature tolerance (37^0 c and 42^0 c)

In testing the yeast for ability to ferment sucrose at 37 and 42℃. The test was carried out by inoculating the yeast strains into test tubes containing 2% sucrose medium and grown in an incubating temperature of 37^0 c and 42^0 c respectively. The growth rate of the yeasts were obtained by measuring the exponential increase in turbidity using a spectrophotometer at 660mm for 0, 24 and 48hours interval. The percentage inhibition or increase in growth rate for each yeast strain was then calculated.

DETERMINATION OF MINERAL CONTENT (VITAMINS)

Five (5g) of the sample was weighed into a silica dish. The dish was previously ignited and cooled before weighing. The dish and content were first gently ignited over Bunsen flame and then in a muffle furnace at 500-55^0 c. The ash content was determined by subtracting the final weight from the initial weight in grams.

Test for protein

A suspension of yeast cells was fixed on a microscopic slide and covered with millions reagent. This was then covered with coverslip and observed under the microscope. Yeast cells containing protein particles react to form a brick red coloration.

RESULTS

Table 1: Cultural, Morphological and Physiological differentiation of Yeast Strains.

Yeast strain	Cultural appearance	Morphology	Ps	My	Glu	Suc	Source	Lac	Source	Mal	Lac	Source
Saccharomyces cerevisiae A	Brownish White with round edge	Ellipsoidal	+	-	+	+	Baker's Yeast	-	Baker's Yeast	+	-	Baker's Yeast
Saccharomyces cerevisiae B	Brownish white smooth and flat	Elongated	-	-	+	+	Banana	-	Bannana	+	-	Bannana
Saccharomyces cerevisiae C	Dull white with rough edges	Ellipsoidal	+	-	+	+	Pawpaw	-	Pawpaw	+	-	Pawpaw
Saccharomyces cerevisiae D	Milky, smooth and flat	Oval	+	-	+	+	Orange	-	Orange	+	-	Orange
Candida tropicalis C	White, rough and raised	Globose	+	+	+	+	Pawpaw	-	Pawpaw	+	-	Pawpaw
Candida tropicalis D	White, rough and raised	Oval	+	+	+	+	Orange	-	Orange	+	-	Orange
Saccharomyces fermentati B	Transparent with smooth edges	Lemon shape	+	-	+	+	Banana	-	Banana	+	-	Banana
Saccharomyces fermentati C	White, rough with round edge	Lemon shape	+	-	+	+	Pawpaw	-	Pawpaw	+	-	Pawpaw

Saccharomyces fermentati D	Transparent with smooth edges	Oval	+	-	+	+	Orange	-	Orange	+	-	Orange

Key : + = positive reactions, Glu = Glucos , – = Negative Reaction, Mal = Maltose
Ps = Pseudo mycelia, My = Mycelia, Suc = Sucrose L = Lactose

Table 2: ESTIMATION OF YEAST PORTENCY

Test performed	**Numbers of cell (%)**									**Colour of cells**
	Saccharomyces cerevisiae				**Candida tropicalis**		**Saccharomyces fermentati**			
	A	B	C	D	C	D	B	C	D	
Viable	98	97	95	95	90	95	98	98	98	Colorless
Dead cells	2	3	5	5	10	15	2	2	2	Blue
Cells with Glycogen	80	75	75	75	80	80	75	80	75	Dark brown
Cells without glycogen	20	25	25	25	20	20	25	20	25	Yellow
Cells with lipid	75	75	75	75	75	75	75	75	75	Red
Cells	Without lipids	25	25	25	25	25	25	25	25	Colorless

Table 3 FLOCCULATION AND ETHANOL TOLERANC OF YEAST STRAINS

Yeast Straw	Flocculation rate ml/10min	Tolerance to ethanol 10%	15%	20%
Saccharomyces cerecvisiae A	0.75	+	+	+
Saccharomyces cerecvisiae B	0.70	+	+	+

Saccharomyces cerecvisiae C	0.75	+	+	+
Saccharomyces cerecvisiae D	0.75	+	+	+
Candida tropicalis C	0.75	+	-	-
Candida tropicalis D	0.70	+	-	-
Saccharomyces fermentati. B	0.40	+	+	-
Saccharomyces fermentati. C	0.50	+	+	-
Saccharomyces fermentati. D	0.50	+	+	-

TABLES 4 TEMPERATURE TOLERANCE FERMENTATION ABILITY OF YEAST AT 37°C AND 42°C WITH 2% W/V GLUCOSE

Yeast strain	37^0c			42^0c		
	24hrs	48hrs	% inhibition	24hrs	48hrs	% inhibition
Saccharomyces cerecvisiae A	0.299	0.303	0	0.106	0.427	32
Saccharomyces cerecvisiae B	0.299	0.303	0	0.106	0.427	32
Saccharomyces cerecvisiae C	0.22	0.111	0.1	0.238	0.405	41
Saccharomyces cerecvisiae D	0.299	0.303	0	0.106	0.427	32
Candida tropicalis C	0.300	0.303	0	0.455	0.225	50
Candida tropicalis D	0.127	0.111	0.01	0.460	0.146	68
Saccharomyces fermentati. B	0.135	0.145	0.1	0.041	0.156	48
Saccharomyces fermentati. C	0.135	0.145	0.1	0.238	0.405	41

Saccharomyces fermentati. D	0.132	0.145	0.1	0.238	0.405	41

TABLE 5 : TEST FOR PROTEIN AND VITAMINS

	PROTEIN (%)		VITAMIN (GR)			
Yeast	Cellwith protein	without protein	Initial weight	Final weight	Estimated content	Source
Saccharomyces cerecvisiae A	95	5	3.00	0.72	2.28	Baker's yeast
Saccharomyces cerecvisiae B	90	10	3.00	0.72	2.28	Banana
Saccharomyces cerecvisiae C	95	5	3.00	0.08	2.20	Pawpaw
Saccharomyces cerecvisiae D	95	5	3.00	0.73	2.27	Orange
Candida tropicalis C	80	20	3.00	0.72	2.28	Pawpaw
Candida tropicalis D	80	20	3.00	0.75	2.25	Orange
Saccharomyces fermentati. B	90	10	3.00	0.84	2.16	Banana
Saccharomyces fermentati. C	90	10	3.00	0.80	2.20	Pawpaw
Saccharomyces fermentati. D	95	5	3.0	0.83	2.17	orange

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

Table 1. shows cultural morphological characteristics of yeast strains. This is in line with the report of Amerine, *et al.*, (2009). who stated that yeast are identified using cultural morphological and physiological properties. According to a him, *saccharomyces cerevisiae* has the ability of assimilating a wide spectrum of sugars as carbon source and its shape ranges from spherical to plump sausage but its typical outline as observed under the microscope is

ellipsoidal. Furthermore, Mrak and Clung (2015) observed that *candida tropicalis* could be identified as non-sporulation film yeast, having an extensive mycelium and capable of fermenting glucose, fructose, maltose and sucrose.

The estimation of yeast potency (Table 2) shows that saccharomyces cerevisiae A Show a high percentage viability of 98% with about 80%, containing glycogen and 75%, containing lipids. Other isolation also showed high viability because none fell below 75%. In this respect, all the isolates compared favorably with the industrial yeast, Amose, *et al.*, (2006) The adequate presence of glycogen and lipids in yeast cells indicate a state of well-nourished cells. More so, the presence of more than 75% of the viable cell containing glycogen and lipids is a very important stable indication of the physiological state of the cells. Thus, local yeast isolates could be used in the same quantity as industrial yeast in any industrial processes .In addition, Pitton, (2014) reported that these reserve materials contribute to wine flavor, implying that isolates from banana pawpaw and orange could serve in the wine producing industry.

The performance of various yeast strains in their flocculation rate and tolerance to various concentrations of ethanol (10%, 15%, 20%) as represented in table 3 shows that *saccharomyces cerevisiae C and saccharomyces cerevisiae* D exhibited the highest flocculation rate compared to the industrial yeast at 0.75ml/10 minutes and were found tolerant to ethanol concentration of 20%. *Saccharomyces fermentati* B was least flocculent, but could tolerate ethanol concentration up to 15%, Whereas *Candida tropicalis* C and D were inhibited by ethanol concentration of 15% but possess a high flocculation rate of 0.75ml/ 10 minutes and 0.7ml/10 minutes respectively. These is supported by the work of Stanbury and Whitaker (2011, who observed that *saccharomyces cerevisiae* survived ethanol concentration up to 20%, hence the choice of this strain for quality wine production. Similarly, the high degree of flocculation obtained for *Sacharomyces cerevisea* B,C,D is an indication that such yeast could be a good raw material for the beer or wine industries as was corroborated by Ameh, *et al*.,(2005), who stated that cells that settles quickly after fermentation, show little or no tendency of the lees rising and mixing with the wine during racking. However the physiology of flocculation is not well understood but seems to involve calcium ions in salt bridges found at the cell wall (Barnet, *et al*., 2013).

It could be established that all strains have the ability to withstand temperature up to 42% with little or no inhibition except *Candida tropicalis* C and D that

showed a percentage inhibition above average (Table 4), Kunkee, (2012) recorded that bread yeast that was used experimentally were characterized by rapid fermentation and formation of amounts of ethanol at high temperature that compare favorably with those produced by wine yeast, *(Saccharomyces cerevisiae*). Though *Saccharomyces cerevisiae* B,C,D and *Saccharomyces* f*ermentati* B,C,D showed a high protein and vitamin content than candida species table 5, as was corroborated by Rose, (2008), who stated that yeast contains a complex combination of B Vitamins and proteins, thus helps to eradicate deficiency diseases such as pellagra and beriberi.

Though *Saccharomyces cerevisiae* B,C,D and *Saccharomyces* f*ermentati* B,C,D showed a high protein and vitamin content than candida species. Data subjected to ANOVA Two factor without replication for the *Saccharomyces* and candida species showed that the p values (0.44) was greater than 0.01 for *Saccharomyces* between species, and for candida species p value (0.38) was greater than 0.01 between species. As a result, there was no significant difference between the *Saccharomyces* and candida species.

Conclusion.

In conclusion local isolates from orange, banana and pawpaw tested in this work possess enough potentials to be used in various industries (breweries, bakeries and pharmaceutical industries among others.)

Recommendation

Since the yeast isolates in this work showed high potency for various industrial applications, it is recommended that production of yeast should be embarked upon, on a pilot scale to ensure the feasibility of the work. it is imperatively clear that its use will surely enhance productivity and minimize cost of production.

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