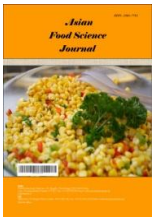

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In Vitro Evaluation of Probiotic Properties of Indigenous Yeasts Isolated from Nigerian Fermented Food Products

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Probiotics are living microbial food supplements which beneficially affect the host by improving the intestinal microbial balance. Before an organism can be designated as probiotic there are certain criteria that must be fulfilled. These include acid and bile tolerance, antimicrobial activity, ability to co-aggregate, hydrophobicity etc. One hundred and eighty one indigenous yeast isolates recovered from various fermented food products of Nigeria were characterized and grouped using phenotypic methods. Forty two selected yeast isolates were identified using molecular method which involved sequencing of D1 and D2 domain of the large subunit of ribosomal DNA. Then nine indigenous *Saccharomyces cerevisiae* were evaluated for their probiotic characteristics such as acid and bile tolerance, transit in simulated gastric and intestinal juices, autoaggregation and hydrophobicity. *Saccharomyces cerevisiae* SC10 was included as a positive control. The *S. cerevisiae* were able to grow in the presence of acidic medium with pH as low as 2 and 3. In the minimum inhibitory concentration test with 0-1% ox bile, all the *S. cerevisiae* tested were able to

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grow. The growth for 3% bile tolerance test ranged from 4.81 to 5.35 log cfu/ml. These isolates were able to survive in simulated gastro-intestinal transit. All the yeast isolates exhibited bile salt deconjugation activity against sodium glycodeoxycholate and were able to grow in the presence of all other bile salts investigated. Autoaggregation ability (an adhesive property) of the indigenous yeast isolates ranged from 89.80% for *S. cerevisiae* BK19 to 99.91% for *S. cerevisiae* OB03. The native yeast isolates also exhibited high percentage hydrophobicity, another adhesive property of probiotics. The values obtained ranged from 31.62 to 83.45% for isolates AG23A and OB 17. These observations indicate that the native yeast isolates from Nigerian fermented foods have the potential of being use as probiotics for making functional foods.

Keywords: Indigenous yeast; fermented foods; probiotic; Nigeria.

1. INTRODUCTION

Fermentation is one of the oldest forms of food processing and preservation in the world [1]. There are three major types of microorganisms that are found in traditional fermented foods and beverages. These are filamentous fungi, yeasts and bacteria [2]. The term "yeast" was derived originally from the Dutch word "gist" which refers to the foam formed during the fermentation of beer wort [3]. Yeasts are microorganisms of great economic interest for their numerous applications in traditional and modern biotechnology [4]. Hayduck was the person who first discovered the inhibitory activity of yeast. Later on, other researchers reported the antagonistic activity of yeasts against other yeasts which involve the production of secondary metabolites [3].

Microbiota is a system conformed by microorganisms that coexist in equilibrium with the host; it performs an important role in protection against infection. The microbiota normally exists in equilibrium with the host, but when this equilibrium is broken many gastrointestinal disorders may develop. This include antibiotic associated diarrheas, ulcers, inflammatory bowel disease, irritable bowel syndrome and some other time cause more severe ailments like colon cancer [5].

Probiotics have been used for hundred of years in the production of cultured dairy products. Probiotics are defined as live microorganisms which when administered in adequate quantity confer a health benefit on the host [6]. Although many yeast species have been isolated from fermented food products, only *Saccharomyces cerevisiae* has GRAS (Generally Recognize as Safe) status. It is the only yeast commercialized as probiotic for human use [2]. Palm wine is an alcoholic beverage produced from the sap of palm tree. Burukutu is an alcoholic beverage produced from fermented sorghum. Ogi-baba is a fermented cereal gruel produce from sorghum while agadagidi is an alcoholic beverage produce from plantain. Despite the fact that different indigenous yeasts have been isolated from various Nigerian traditional fermented food products, their probiotic attributes are scarcely reported in the literature. Therefore, the objective of the current study is to investigate the probiotic properties of yeasts isolated from selected Nigerian traditional fermented foods.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification of Yeast Isolates

Ten samples of traditional fermented foods like palm wine, burukutu and ogi-baba were collected from local producers immediately after production and transported to the laboratory in sterile containers. Ten samples of agadagidi were produced in the laboratory by following the traditional method. The food samples were stored under refrigeration until used in isolation of yeasts. The food samples were serially diluted in sterile distilled water and appropriate dilutions (10^{-5}) were plated on Potato Dextrose Agar (PDA) supplemented with antibiotic such as chloramphenicol and streptomycin (3µg/ml). The yeast isolates were purified by repeated streaking on PDA and later identified by cultural, morphological, physiological and molecular methods [7].

2.2 Survival Tolerance Studies

The selected test organisms obtained from various indigenous fermented food products were used in this experiment. *Saccharomyces cerevisiae* SC10 obtained from the laboratory in Palampur, India was incorporated as a positive control.

2.3 Acid Tolerance Test

Potato dextrose broth (PDB) was adjusted to pH 2 and 3 using 5.0M HCL. Inoculation of approximately 10^{10} cfu/ml of each yeast strain was done into the modified PDB and incubation was carried out at 37°C for 3 hours. Samples were withdrawn at one hour interval and then spread plated on PDA plates. The initial plates count at 0 hours was compared with that of 3 hours to determine acid tolerance [8].

2.4 Bile Tolerance Tests

2.4.1 Minimum inhibitory concentration method

Twenty microliter of each culture of *S. cerevisiae* were spotted in Potato dextrose agar containing 0.1 – 1% (w/v) ox bile. The cultures were incubated aerobically for 48 hours and minimum inhibitory concentration (MIC) was taken as the smallest concentration completely inhibiting the development of spots [8].

2.4.2 Plate count method

In order to study bile tolerance of the yeast isolates, ox bile was used using plate count method. Potato dextrose broth (PDB) containing 3% (w/v) of ox bile was adjusted to pH 5.8 by 5.0M HCl. The PDB containing bile salts were inoculated with 10^{10} cfu/ml and incubated at 28°C for 8 hours. Viable count (cfu/mL) was measured at 0, 4 and 8 hours interval on PDA and was incubated at 28°C aerobically for 48 hours [9].

2.5 Production of Replicated Gastric and Intestinal Juices

In order to prepare gastric juice, pepsin was dissolved in sterilized normal saline (0.85% w/v) to a finishing concentration of 3g/l. In order to prepare intestinal juice pancrease was dissolve to a finishing concentration of 1g/l and its pH adjusted to pH 8. Millipore filters were used for the sterilization of the juices obtained. Then the yeast cultures were inoculated into the juices separately and incubation was done at 37°C for 4 hours [9].

2.6 Production of Washed Yeast Cell Preparation

The yeasts were cultured in PDB for 48 hours at 28°C. Then centrifugation at 4000 xg at 4°C for 10 minutes was done. The yeasts harvested were mixed with phosphate buffered saline (PBS) and washed thrice at pH7. The pellets obtained were mixed with normal saline and number of cells that are viable was determined by spread plating method [9].

2.7 Assay of Upper Gastrointestinal Transit Tolerance

The yeast cultures were inoculated (10^9cfu/ml) into simulated gastric juice (pH2.0 and 3.0). Same volume of 0.2 ml cleaned cell preparation was added to 0.3 ml of sodium chloride (0.5% w/v) and 1.0 ml of tested gastric juice (pH 3 or pH 2). 0.1 mL of sample was taken at 30 minutes interval up to 240 minutes and spread plated on PDA and viable count determined [6]. The intestinal transit tolerance was determined by taking 0.1 mL of each isolate and added to 1 mL of replicated intestinal fluid (pH 8.0) and later mixed with 0.3 ml of sodium chloride (0.5% w/v). The cultures were later incubated aerobically at 28°C and viable counts determined after 0, 1, 4 and 8 hours of incubation. The yeast isolates were studied for growth in potato dextrose broth (PDB). Fresh cultures of yeast isolates (1%) were incubated in the PDB and incubated at 28°C and monitored for growth at 620nm with a spectrophotometer on an hourly basis for 9 hours or until absorbance increase by 0.3 unit [9].

2.8 Hydrophobicity of the Yeast Isolates

In order to determine cell exterior hydrophobicity, connection of microbes to hydrocarbons which are n-hexadecane, xylene and toluene was investigated. The yeast cultures were grown in PDB for 48 hours at 28°C. The cells were harvested by centrifugation and then washed two times with phosphate buffer with pH 6.5 and remixed again in the same phosphate buffer. 3 ml of yeast cells was mixed with 0.6ml of n-hexadecane, xylene and toluene separately and vortexed for 120s. The separation of the two phases was carried out at 28°C for 1hour. The water containing phase was removed cautiously and absorbance was measured at 250 nm. Percentage hydrophobicity was measured according to the following equation/formula:

$$\% H = [(A_o - A]/A_o] \times 100$$

Where A_o was the absorbance value before extraction and A was absorbance value after extraction with hydrocarbons [2].

2.9 Autoaggregation Ability of Yeast Isolates

Yeast isolates were cultured in PDB and incubated at 28°C for 48 hours. Potato dextrose broth (PDB) containing the yeast cells was centrifuged and then re-suspended in phosphate buffered saline (PBS). 5ml of yeast cells was obtained and incubation was done at 28°C for 20 hours. After the culture had settled down, 3 mL of the upper part of suspension was carefully obtained into sterile tubes and the optical density (O.D) read at 600 nm. Autoaggregation strength of the yeast isolates was estimated as percentage:

(upper-suspensionO.D/ total yeast-suspensionO.D) X 100 [2].

2.10 Bile Salt Deconjugation Activity of the Yeast Isolates

Potato dextrose agar (PDA) containing 0.5% (w/v) of different sodium salts was prepared (sodium glycodeoxycholate, sodium taurodeoxycholate hydrate, sodium taurocholate, sodium glycolate and sodium glycolate hydrate). The plates were spot inoculated and incubated at 28°C for 3 days. Positive result was taken as the appearance of precipitate around the yeast colonies [8].

2.11 β-Galactosidase Enzyme Production by the Yeast Isolates

Approximately 2.383 mg of isopropyl β-D-l-thiogalactopyranoside (IPTG) was dissolved in 100 ml of sterile double distilled water to prepare 100mM solution of IPTG. Accordingly, 50 mg/mL solution of x-gal was also prepared in N, N-dimethyl formamide. The two solutions were sterilized by filtration through 0.2 μm (Millipore) filters. To detect the cultures producing β-Galactosidase, 100μL of IPTG and 20 μL of x-gal solutions, were poured on the surface of PDA plated aseptically. Then the yeast cultures were inoculated on the Petri dishes and incubation done at 28°C for 48 hours. The colonies producing this enzyme are blue in colour while non-producers are white in colour [8].

3. RESULTS

The yeasts grew to different extent in the acidic medium. The viable count recorded for the yeasts in the medium where the pH has been adjusted to 2 ranged between 5.4 and 7.5 log_{10}CFU/ml. The highest viable count recorded was 6.2 log_{10}CFU/ml for isolates AGG23A and OBB03A after three hours of incubation (Table 1). The yeasts also grew to different extent in the acidic medium when the pH was adjusted to 3 and incubation was done for 3 hours. Two *Saccharomyces cerevisiae* trains BKT07 and OBB17 had the highest growth of 7.2 log_{10}CFU/ml while *Saccharomyces cerevisiae* SC01 had the lowest growth of 6.1 log_{10}CFU/ml at the end of 3 hours incubation (Table 2).

All the yeast isolates were exposed to different concentration of ox bile ranging from 0.1% to 1.0%. All the yeast isolates grew at these various concentrations (Table 3). This indicated that all *Saccharomyces cerevisiae* strains were tolerant to ox bile. The yeast isolates demonstrated viable count to different extent in the potato dextrose broth supplemented with 3% ox bile with pH adjusted to 5.8. At the end of 8 hours of incubation, *Saccharomyces cerevisiae* OBB17 had the highest viable count of 5.35log_{10}cfu/ml while *Saccharomyces cerevisiae* BKT19 had least viable count of 4.81 log_{10}cfu/ml (Table 4).

In order to evaluate the capacity of the yeast isolates to survive in the stomach, gastric transit and intestinal tests were carried out. The media pH was adjusted to 2 and 3 and length of incubation was 4 hours. At pH 2, zero hour viable count ranged between 8.31 and 9.2 log_{10}cfu/ml. At the end of 4 hours of incubation, the highest viable count was 9.50 log_{10}cfu/ml. When the pH was adjusted to 3, the viable counts ranged between 7.40 and 8.40 log_{10}cfu/ml at the end of 4 hours of incubation (Table 5). For an organism to be designated as probiotic it must survive in the intestine. During the transit in the intestine the yeast must maintain a high viable count. The viable count of these indigenous yeast isolate was very high and it ranged between 7.27 and 8.93 log_{10}cfu/ml (Table 6).

The adhesive property was measured indirectly as the adhesion of the isolates to hydrocarbons such as xylene, toluene and N-hexadecane. *Saccharomyces cerevisiae* OBB17 demonstrated a high percentage of hydrophobicity (83.45%). The yeast that had lowest adhesion against xylene was *Saccharomyces cerevisiae* AGG23A with 35.66% (Table 7). Autoaggregation is another way of measuring adhesive property of the yeasts. *Saccharomyces cerevisiae* OBB03A and *Saccharomyces cerevisiae* SC01

demonstrated the highest autoaggregation ability (99.91%). *Saccharomyces cerevisiae* BK19 was the only yeast that had 89.80% autoaggregation (Table 8). All the indigenous yeast isolates grew on all the bile salts concentration used in this work (Table 9) with the exception of SC10 which did not grow on sodium glycolate. Sodium glycodeoxycholate was the only bile salt that was deconjugated by all the isolates.

For an organism to be designated as probiotics it must produce some enzymes which will help directly during digestion. Among these enzymes one of most important is β-galactosidase as shown in Table 10. *Saccharomyces cerevisiae* OBB17 had 0.537M while *Saccharomyces cerevisiae* AG23A had 48.052M.

4. DISCUSSION

One method that is described for selecting potential probiotic microbes is the *in vitro* method which relied on the ability of microbes to stay alive in the tested situation of the digestive tract as this condition must be fulfilled before an organism is designated as probiotic [8]. The indigenous yeast isolates tested in the work were able to stay alive in the low acidic pH 2 and pH 3. The results obtained are comparable to those obtained by Sourabh et al. [8] who reported a decrease in viability of 2.710 to 4.120 log cfu/ml (pH 2) and 0.88 to 3.06 log cfu/ml (pH 3) after 3 hours of exposure. In another study, five yeast isolates from *Idle* batter and two isolates from *Jalebi* batter demonstrated a high acid tolerance at pH 2 after exposure for up to 3 hours [2]. These authors further stated that after mounting the contact time to 5 hours there was no significant reduction in the endurance. This therefore shows that indigenous yeast isolates with proven acid tolerance could be good candidate as probiotics.

In this present study all the nine indigenous yeast isolates were exposed to different concentration of bile. The results obtained showed that every yeast isolate survives at the various concentration tested. The yeast isolates were there after subjected to growth in 3% bile concentration with pH adjusted to 5.8. After 8 hours incubation all the yeast isolates were still viable. Garcia-Hermandez et al. [4] reported the capability of avian yeast to survive under stressful condition of high concentration of bile salts.

Bile salts are dangerous to existing cells, and they destroy the constitution of the cell outer most layer. Studies that have been carried out before showed that the standard quantity of bile salt in the intestine of human being was around 0.30% (w/v) [4]. Consequently, the tolerance of yeast strains to bile salt was thought to be an important asset for chosen novel strains which can stay alive in the intestine, and the quantity has been used for chosen strains that are bile tolerant [6].

For any microorganism to be designated as probiotics, it should be capable of surviving the gastrointestinal tract. The capability of the indigenous yeast isolates to survive in these types of environmental conditions was evaluated. Perriocone et al. [10] stated that there was no important change in the cell counts in gastric juice, in bile salts and pancreatic fluid.

Hydrophobicity of potential yeast isolates is an indirect method of measuring the adhesive properties of probiotics attributes. In order to evaluate this trait, the ability of the yeast isolates to bind to hexadecane, xylene, toluene and tolerance was conducted. The results showed that all the yeast isolates are hydrophobic. Perriocone et al. [10] also reported that only 9 isolates were hydrophobic using hexadecane as the binding agent. Another indirect method of measuring adhesion is the ability to form biofilm [10]. But this parameter was not investigated in the study since it had not been investigated by 99% of work recently reported.

The autoaggregation is another indirect way of measuring adhesive properties of probiotic yeasts. Autoaggregation (%) ability of the yeast isolate was evaluated at different time interval. The autoaggregation ability obtained from the present study was between 89.80% for *Saccharomyces cerevisiae* BKT19 and 91.91% in *Saccharomyces cerevisiae* AGG23A, OBB03A and SC01. Syal and Vohra [2] stated autoaggregation of above 80% after 3 hours of incubation in their study. They further stated that the autoaggregation ability for all isolates increased to 95-100% after 20 hours of incubation.

Table 1. Survival tolerance of the indigenous *Saccharomyces cerevisiae* (log_{10} CFU/ml) (pH 2) isolated from fermented food products

S/N	Isolates	Time (hours)			
		0	1	2	3
1	*Saccharomyces cerevisiae* PAW02	7.5 ± 0.02^{a}	6.6 ± 0.03^{a}	6.2 ± 0.06^{b}	6.1 ± 0.05^{a}
2	*Saccharomyces cerevisiae* PAW24	7.4 ± 0.05^{ab}	6.5 ± 0.06^{b}	6.4 ± 0.06^{a}	6.0 ± 0.09^{bc}
3	*Saccharomyces cerevisiae* BKT07	6.7 ± 0.04^{b}	6.5 ± 0.03^{bc}	6.2 ± 0.03^{ab}	6.0 ± 0.12^{b}
4	*Saccharomyces cerevisiae* BKT19	6.7 ± 0.05^{c}	6.5 ± 0.04^{c}	5.4 ± 0.03^{ac}	6.0 ± 0.05^{c}
5	*Saccharomyces cerevisiae* AGG23A	7.6 ± 0.06^{d}	6.5 ± 0.00^{d}	6.1 ± 0.05^{ab}	6.2 ± 0.09^{bc}
6	*Saccharomyces cerevisiae* AGG08	7.5 ± 0.04^{cd}	6.6 ± 0.03^{cd}	6.2 ± 0.04^{de}	6.1 ± 0.03^{ab}
7	*Saccharomyces cerevisiae* OBB17	7.3 ± 0.05^{a}	6.4 ± 0.05^{a}	6.3 ± 0.05^{d}	6.1 ± 0.04^{ac}
8	*Saccharomyces cerevisiae* OBB03A	7.4 ± 0.06^{c}	5.9 ± 0.01^{ab}	6.4 ± 0.12^{ab}	6.2 ± 0.05^{de}
9	*Saccharomyces cerevisiae* SC01	7.5 ± 0.05^{b}	6.5 ± 0.02^{ac}	6.2 ± 0.21^{bc}	6.0 ± 0.06^{d}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Table 2. Acid tolerance of the indigenous *Saccharomyces cerevisiae* (log_{10}CFU/ml) (pH 3) isolated from fermented food products

S/N	Isolates	Time (hours)			
		0	1	2	3
1	*Saccharomyces cerevisiae* PAW02	6.9 ± 0.05^{a}	7.3 ± 0.04^{a}	6.8 ± 0.15^{a}	7.0 ± 0.01^{c}
2	*Saccharomyces cerevisiae* PAW24	6.5 ± 0.06^{b}	7.7 ± 0.05^{a}	7.1 ± 0.19^{bc}	6.7 ± 0.05^{b}
3	*Saccharomyces cerevisiae* BKT07	6.7 ± 0.05^{ab}	6.9 ± 0.09^{ab}	7.0 ± 0.05^{ab}	7.2 ± 0.04^{a}
4	*Saccharomyces cerevisiae* BKT19	6.6 ± 0.06^{bc}	6.6 ± 0.07^{c}	6.4 ± 0.04^{a}	6.2 ± 0.04^{cd}
5	*Saccharomyces cerevisiae* AGG23A	6.8 ± 0.05^{c}	7.2 ± 0.04^{de}	6.7 ± 0.06^{b}	7.1 ± 0.02^{de}
6	*Saccharomyces cerevisiae* AGG08	6.6 ± 0.12^{d}	7.8 ± 0.06^{a}	7.2 ± 0.04^{a}	6.8 ± 0.06^{a}
7	*Saccharomyces cerevisiae* OBB17	6.8 ± 0.05^{de}	7.0 ± 0.04^{a}	7.0 ± 0.03^{a}	7.2 ± 0.03^{b}
8	*Saccharomyces cerevisiae* OBB03A	6.7 ± 0.05^{a}	6.7 ± 0.05^{a}	6.5 ± 0.05^{b}	6.2 ± 0.02^{a}
9	*Saccharomyces cerevisiae* SC01	6.6 ± 0.07^{ab}	6.6 ± 0.06^{a}	6.4 ± 0.02^{a}	6.1 ± 0.02^{ab}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Table 3. Bile tolerance test for the indigenous *Saccharomyces cerevisiae* isolated from fermented food products

Isolates	Concentration of Bile (%)										
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	No bile (Control)
S. cerevisiae PAW02	+	+	+	+	+	+	+	+	+	+	+
S. cerevisiae PAW24	+	+	+	+	+	+	+	+	+	+	+
S. cerevisiae BKT07	+	+	+	+	+	+	+	+	+	+	+
S. cerevisiae BKT19	+	+	+	+	+	+	+	+	+	+	+
S. cerevisiae AGG23A	+	+	+	+	+	+	+	+	+	+	+
S. cerevisiae AGG08	+	+	+	+	+	+	+	+	+	+	+
S. cerevisiae OBB17	+	+	+	+	+	+	+	+	+	+	+
S. cerevisiae OBB03A	+	+	+	+	+	+	+	+	+	+	+
S. cerevisiae SC01	+	+	+	+	+	+	+	+	+	+	+

KEY:+=growth in the medium

Table 4. Tolerance to 3% bile concentration by the indigenous *Saccharomyces cerevisiae* isolated from fermented food products

S/N	Isolates	Time (hours)/log_{10}CFU/ml						
		0	2	4	5	6	7	8
1	*S. cerevisiae* PAW02	6.27±0.02a	5.76±0.05^{a}	5.48±0.02^{a}	5.47±0.04^{a}	5.46±0.04^{a}	5.36±0.04^{a}	5.22±0.15^{a}
2	*S. cerevisiae* PAW24	6.91±0.06^{b}	5.70±0.02^{b}	5.64±0.05^{b}	5.49±0.04^{a}	5.30±0.04^{a}	5.22±0.03^{a}	5.09±0.12^{a}
3	*S. cerevisiae* BKT07	5.95±0.05^{a}	5.81±0.06^{c}	5.70±0.09^{c}	5.58±0.05^{b}	5.12±0.05^{b}	5.12±0.05^{b}	4.91±0.01^{b}
4	*S. cerevisiae* BKT19	5.47±0.02^{b}	5.76±0.05^{d}	5.64±0.06^{b}	5.47±0.04^{a}	5.38±0.03^{a}	5.15±0.06^{c}	4.81±0.05^{b}
5	*S.cerevisiae* AGG23A	7.51±0.05^{c}	6.30±0.02^{e}	5.90±0.09^{c}	5.48±0.04^{a}	5.40±0.04^{a}	5.35±0.05^{a}	5.30±0.06^{b}
6	*S. cerevisiae* AGG08	7.52±0.06^{de}	6.20±0.02^{a}	5.96±0.09^{c}	5.15±0.06^{bc}	5.33±0.04^{a}	4.94±0.06^{a}	4.92±0.03^{c}
7	*S. cerevisiae* OBB17	6.85±0.09^{d}	5.80±0.09^{ab}	5.65±0.05^{b}	5.50±0.05^{a}	5.45±0.03^{ab}	5.38±0.05^{a}	5.35±0.06^{bc}
8	*S. cerevisiae* OBB03A	5.95±0.06^{e}	5.75±0.05^{cd}	5.65±0.06^{b}	5.55±0.06^{bc}	5.50±0.04^{a}	5.39±0.05^{b}	5.34±0.04^{c}
9	*S. cerevisiae* SC01	6.30±0.04^{cd}	5.81±0.09^{cd}	5.65±0.05^{b}	5.59±0.06^{bc}	5.55±0.06^{b}	5.30±0.05^{b}	5.25±0.05^{b}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Table 5. Gastric transit tests viable count for the indigenous *Saccharomyces cerevisiae* isolated from fermented food products

S/N	Isolates	pH 2					pH 3				
		Incubation periods (hour)/ Viable Count (logCFU/ml)									
		0	1	2	3	4	0	1	2	3	4
1	*S.cerevisiae* PAW02	8.31±0.05^{a}	9.06±0.05^{a}	9.36±0.04^{a}	9.43±0.04^{a}	9.50±0.05^{a}	8.86±0.05^{a}	8.43±0.04^{a}	8.32±0.02^{a}	8.30±0.04^{a}	7.48±0.04^{a}
2	*S.cerevisiae* PAW24	8.38±0.03^{b}	8.33±0.02^{b}	8.27±0.03^{a}	8.05±0.05^{b}	6.80±0.06^{b}	8.46±0.04^{b}	8.40±0.03^{a}	8.32±0.03^{a}	8.30±0.03^{b}	8.08±0.02^{b}
3	*S.cerevisiae* BKT07	9.15±0.04^{b}	8.02±0.04^{b}	7.68±0.05^{b}	7.63±0.06^{b}	7.49±0.04^{c}	9.76±0.06^{a}	8.95±0.05^{b}	8.52±0.05^{b}	8.50±0.05^{c}	8.35±0.03^{c}
4	*S.cerevisiae* BKT19	8.61±0.06^{a}	8.68±0.05^{a}	8.71±0.06^{b}	8.82±0.09^{b}	8.93±0.09^{a}	8.75±0.06^{a}	8.51±0.06^{b}	8.45±0.06^{b}	8.42±0.04^{a}	8.40±0.04^{a}
5	*S.cerevisiae*AGG23A	8.50±0.05^{a}	8.35±0.04^{b}	8.30±0.02^{c}	8.20±0.02^{c}	8.15±0.02^{c}	8.50±0.05^{a}	8.47±0.04^{a}	8.45±0.06^{b}	8.35±0.03^{b}	8.25±0.02^{b}
6	*S.cerevisiae* AGG08	9.20±0.03^{b}	8.05±0.05^{a}	7.70±0.07^{d}	7.60±0.06^{b}	7.50±0.04^{c}	9.55±0.06^{a}	9.07±0.02^{c}	8.70±0.07^{c}	8.50±0.05^{c}	8.40±0.02^{b}
7	*S. cerevisiae* OBB17	8.40±0.04^{c}	8.30±0.06^{c}	8.25±0.04^{c}	8.20±0.04^{a}	8.00±0.02^{d}	8.55±0.09^{c}	8.50±0.05^{b}	7.80±0.06^{b}	7.50±0.06^{d}	7.40±0.04^{a}
8	*S.cerevisiae* OBB03A	8.50±0.05^{d}	8.40±0.05^{a}	8.30±0.03^{c}	8.20±0.04^{a}	8.14±0.04^{bc}	8.49±0.02^{d}	8.40±0.04^{a}	8.35±0.04^{d}	8.28±0.04^{a}	8.0±0.03^{d}
9	*S. cerevisiae* SC01	8.60±0.04^{b}	8.50±0.19^{b}	8.40±0.02^{d}	8.25±0.05^{b}	8.10±0.04^{b}	8.00±0.04^{d}	7.90±0.06^{b}	7.85±0.05^{b}	7.80±0.06^{d}	7.50±0.05^{e}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Table 6. Intestinal transit tests for the indigenous *Saccharomyces cerevisiae* isolated from fermented food products

S/N	Isolates	Incubation periods (hours)/Viable count: (logcfu/ml)			
		0	1	4	8
1	*S. cerevisiae* PAW02	8.90±0.05^{a}	8.88±0.05^{a}	8.63±0.05^{a}	8.32±0.03^{a}
2	*S. cerevisiae* PAW24	7.97±0.06^{b}	7.94±0.06^{b}	7.49±0.04^{b}	7.37±0.04^{b}
3	*S. cerevisiae* BKT07	8.44±0.04^{c}	8.41±0.04^{c}	8.36±0.03^{c}	8.29±0.02^{c}
4	*S. cerevisiae* BKT19	8.96±0.06^{b}	8.93±0.06^{b}	8.18±0.03^{d}	8.04±0.03^{a}
5	*S. cerevisiae* AGG23A	8.45±0.03^{d}	8.40±0.04^{c}	8.37±0.03^{c}	8.30±0.03^{d}
6	*S. cerevisiae* AGG08	8.85±0.06^{b}	8.80±0.05^{a}	8.74±0.06^{e}	8.60±0.05^{a}
7	*S. cerevisiae* OBB17	8.54±0.05^{a}	8.50±0.06^{b}	8.47±0.04^{b}	8.40±0.03^{b}
8	*S. cerevisiae* OBB03A	7.50±0.04^{c}	7.48±0.04^{c}	7.45±0.03^{c}	7.30±0.04^{b}
9	*S. cerevisiae* SC01	8.50±0.05^{a}	8.45±0.04^{c}	8.30±0.03^{c}	8.20±0.02^{c}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Table 7. Hydrophobicity/Microbial adhesion to hydrocarbons by the indigenous *Saccharomyces cerevisiae* isolated from fermented food products

S/N	Isolates	Percentage hydrophobicity (%)		
		Xylene	Toluene	N-hexadecane
1	*S. cerevisiae* PAW02	61.62±0.05^{a}	47.37±0.04^{a}	60.49±0.04^{a}
2	*S. cerevisiae* PAW24	61.80±0.06^{b}	55.85±0.06^{b}	70.55±0.05^{b}
3	*S. cerevisiae* BKT07	42.57±0.05^{a}	57.50±0.05^{c}	57.73±0.06^{c}
4	*S. cerevisiae* BKT19	67.54±0.05^{a}	60.68±0.06^{b}	57.05±0.02^{d}
5	*S. cerevisiae* AGG23A	35.66±0.06^{b}	42.28±0.02^{d}	51.17±0.01^{e}
6	*S. cerevisiae* AGG08	58.00±0.12^{c}	24.21±0.03^{d}	54.48±0.04^{a}
7	*S. cerevisiae* OBB17	76.68±0.06^{b}	64.50±0.05^{c}	83.45±0.04^{a}
8	*S. cerevisiae* OBB03A	63.35±0.05^{a}	76.49±0.04^{a}	66.37±0.05^{b}
9	*S. cerevisiae* SC01	66.82±0.06^{b}	55.50±0.03^{d}	61.08±0.02^{d}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Table 8. Autoaggregation ability of the indigenous *Saccharomyces cerevisiae* isolated from fermented food products

S/N	Isolates	Optical density		Autoaggregation (%)
		Upper suspension	Total suspension	
1	*S. cerevisiae* PAW02	0.039±0.01^{a}	0.813±0.01^{a}	95.20±0.04^{a}
2	*S. cerevisiae* PAW24	0.018±0.02^{b}	1.021±0.03^{b}	98.24±0.04^{a}
3	*S. cerevisiae* BKT07	0.023±0.02^{b}	0.957±0.02^{c}	97.60±0.05^{b}
4	*S. cerevisiae* BKT19	0.111±0.01^{a}	1.093±0.03^{b}	89.80±0.05^{b}
5	*S. cerevisiae* AGG23A	0.003±0.00^{c}	0.858±0.02^{c}	99.91±0.06^{c}
6	*S. cerevisiae* AGG08	0.036±0.03^{d}	1.115±0.03^{b}	96.77±0.06^{c}
7	*S. cerevisiae* OBB17	0.004±0.00^{e}	1.093±0.03^{b}	99.63±0.05^{b}
8	*S. cerevisiae* OBB03A	0.001±0.00^{e}	1.127±0.03^{b}	99.91±0.06^{b}
9	*S. cerevisiae* SC01	0.016±0.04^{ab}	0.846±0.02^{c}	99.91±0.06^{b}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

Table 9. Bile salts deconjugation activity of the indigenous yeasts isolated from fermented food products

S/N	Isolates	Bile salts				
		Sodium glycodeoxycholate	Sodium Taurodeoxycholate hydrate	Sodium Taurocholate	Sodium glycolate	Sodium Glycolate hydrate
1	*S. cerevisiae* PAW02	+	-	-	-	-
2	*S. cerevisiae* PAW24	+	-	-	-	-
3	*S. cerevisiae* BKT07	+	-	-	-	-
4	*S. cerevisiae* BKT19	+	-	-	-	-
5	*S. cerevisiae* AGG23A	+	-	-	-	-
6	*S. cerevisiae* AGG08	+	-	-	-	-
7	*S. cerevisiae* OBB17	+	-	-	-	-
8	*S. cerevisiae* OBB03A	+	-	-	-	-
9	*S. cerevisiae* SC01	+	-	-	-	-

Key:+=growth;-=no growth

Table 10. β-galactosidase Activity of the Indigenous Yeasts Isolated from Fermented Food Products

S/N	Isolates	Optical Density			β-glalactosidase Activity (Miller)
		Initial	Final		
		560 nm	420 nm	560 nm	
1	*S. cerevisiae* PAW02	1.256 ± 0.05^{a}	0.187 ± 0.05^{a}	0.021 ± 0.01^{a}	9.037 ± 0.03^{a}
2	*S. cerevisiae* PAW24	0.880 ± 0.06^{b}	0.185 ± 0.05^{a}	0.009 ± 0.02^{b}	12.822 ± 0.04^{b}
3	*S. cerevisiae* BKT07	0.500 ± 0.05^{a}	0.270 ± 0.02^{b}	0.092 ± 0.02^{b}	14.533 ± 0.05^{c}
4	*S. cerevisiae* BKT19	0.954 ± 0.06^{b}	0.208 ± 0.02^{b}	0.022 ± 0.04^{c}	11.845 ± 0.04^{b}
5	*S. cerevisiae* AGG23A	0.154 ± 0.01^{c}	0.230 ± 0.02^{b}	0.068 ± 0.05^{d}	48.052 ± 0.06^{d}
6	*S. cerevisiae* AGG08	0.858 ± 0.06^{b}	0.195 ± 0.05^{a}	0.014 ± 0.06^{ab}	13.248 ± 0.05^{c}
7	*S. cerevisiae* OBB17	0.822 ± 0.06^{b}	0.210 ± 0.06^{c}	0.018 ± 0.06^{ab}	0.537 ± 0.03^{a}
8	*S. cerevisiae* OBB03A	1.059 ± 0.05^{a}	0.242 ± 0.05^{a}	0.043 ± 0.04^{c}	10.497 ± 0.04^{b}
9	*S. cerevisiae* SC01	1.290 ± 0.05^{a}	0.220 ± 0.04^{d}	0.048 ± 0.04^{c}	7.0284 ± 0.02^{d}

Mean values of two replicates with different superscript down the column are significantly different at $\alpha_{0.05}$

5. CONCLUSION

One of the significant ways by which probiotics beneficially influence the correct condition of the host is by enzymatic actions that advance the nutrients consumption in the intestine (Sourabh et al., 2011). Although these authors could not isolate yeast with β-galactosidase activity, in this present work all the yeast isolates demonstrated β-galactosidase activity. This is a very interesting result because these isolates will be able to hydrolyze lactose.

Deconjugation is one of the major actions of intestinal microbes included by the World Health Organization (WHO) professionals for selection of probiotic organisms. There is development of white halo or opaque zones surrounding the colony which resulted from the release of free bile acids upon deconjugation of bile salts that was added and this is regarded as deconjugation activity of an organism (Sourabh et al., 2011). All the 9 yeast isolates of this study were able to demonstrate deconjugation activity against sodium glycodeoxycholate but were only able to grow in the presence of all other sodium salts tested. Deshpande et al. (2014) reported the ability of *Lactobacillus casei* to deconjugate bile salts. It was found that isolated *Lactobacilli* cells were able to deconjugate the bile salt which was observed on the surface of colonies in the form of white precipitate. Rasti et al. (2011) reported deconjugation activity of *Bifidobacterium pseudocatenulatum*. It was reported that *B. pseudocatenulatum* had ability to deconjugate 100% of the bile salts in 0.25mM of all types at pH 5.7. They further stated that at pH 6.2 the percentage of deconjugation of all types of bile

salts (0.25mM) induced by *B. pseudocatenulatum* G4 after 24 hours was 100%.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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