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(RESEARCH ARTICLE)

# *In vitro* assessment of probiotic potentials of yeast strains isolated from a cereal-based (Ogi)) traditional fermented food

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## Abstract

This present research focused on the isolation of yeast strains from fermented African cereal-based food with promising probiotic attributes. Yeast isolates were obtained from a locally produced cereal-based food "Ogi" also known as Akamu. The yeast was evaluated for their stress tolerance (pH, bile, temperature, NaCl, gastric juice), adhesion properties (auto-aggregation, hydrophobicity) and susceptibility to antibiotics. The strains performed well at 37 °C and exhibited strong ability to tolerate acid (pH 3) and bile salt (1.5%) environment with a percentage survival range of 60-82.61% and 62.96-83.67% respectively. *Candida tropicalis* (A4) gave the highest salt tolerance at 1% (89.29) and 4% (92.86), while *Saccharomyces cerevisiae* (A2) showed greater ability to survive the stimulated gastrointestinal conditions at 42.86%. The strains exhibited high auto-aggregation (68.8-94.9%) and a good hydrophobicity to chloroform and toluene. Susceptibility profile of the yeast strains to antibacterial antibiotics showed that all the yeasts presented a resistance pattern. These results indicate that *C. tropicalis* and *S. cerevisiae* both have the capability to serve as probiotic agents and can be proposed for various applications. Also, fermented cereal-based Ogi with its wide array of health benefits can serve as a carrier for the delivery of probiotics to both animals and humans as food supplements.

**Keywords:** Probiotics; Yeast; Health attributes; Ogi; Adhesion; Antibiotics

## 1. Introduction

The description of probiotics as a Greek word that denotes 'for life' has progressed over time and is currently being described as "live microorganisms that, when dispensed in sufficient quantity, bestow health advantages on the host" [1].Probiotics are considered beneficial because they've been proven to confer certain beneficial effects, like inhibiting the growth of pathogenic bacteria,boosting immune functions, altering the stability of the intestinal microflora, and increasing resistance to infection [2].Strains with probiotic potential are required to undergo screening for their ability to withstand the surroundings of the gastrointestinal tract [3]. These properties required include tolerance to low pH and bile, ability to adhere to the epithelial cell of the gut, limit pathogen adherence, production of acids, bacteriocins and hydrogen peroxide, against pathogenic growth [4, 5].

Fermented foods have various health benefits attributed to them, which are a result of the probiotic properties of the fermentative microorganisms [6].In Africa, fermentation is a cultural activity practiced locally, and the foods produced usually serve as the major source of nutrition within the indigenous communities. These microorganisms liable for the fermentation of African foods have various properties, some of which are associated with probiotic qualities; although the dose needed to confer health advantages as well as their efficacy have not been properly defined [7].

Contrary to previous knowledge that lactic acid bacteria are the only probiotics, yeasts have also been identified as probiotics, and theseare the major microorganisms that occur during fermentations [8, 9].Various essential

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fermentation metabolites, minerals and enzymes are produced by yeast cells, which makes them useful and can be utilized as biotherapeutics due to their promising antimicrobial, antioxidant, and anticancer properties; they are also known to be antagonistic against fungi and bacteria due to their ability to produce VOC's (volitile organic compounds), mycocins, and antimicrobials [10,11].

Although there has been a lot more focus on bacteria as probiotics,yeasts have shown great potential for the evolution of new probiotics, which is evident in the report given by a number of researchers on yeast strains from fermented foods both *in vitro* and *in vivo* studies for their possibility as probiotics [12, 13, 14, 15, 16]. Despite these reports on the promising potential of several probiotic strains, there's still continuous research for new potential probiotic strains with more standard specifications due to their importance in various human and animal applications [17, 18, 19, 20].

Ogi (fermentedcereal gruel) is a very significant cereal-based Nigerian traditional fermented food that could serve as a source of yeast due to its characteristic diversity of yeasts. In the current study, we evaluate the *in vitro* probiotic attributes of yeast strains obtained from Ogi

## 2. Material and methods

### 2.1. Sample collection and isolation

Samples of locally made fermented cereal pudding (Ogi/Akamu) were collected from different local producers at Eke-Awka market, Anambra state, Nigeria. It was collected and stored in a sterile Ziploc bag and transferred to a Microbiology Laboratory where it was analyzed within one hour of collection.

The sample (Ogi) was serially diluted using a 10-fold dilution method and was inoculated into YDA media using spread plate method and was incubated at 37 °C for a period of 48 h, then physical growth was observed on the plates. The organisms were sub-cultured into YDA plate to obtain a pure cultures, which were then stored in agar slants for subsequent use.

### 2.2. Preparation of Cell Suspension

A 24 h grown culture of the isolated yeast strains were inoculated in 10 mL YPD broth and incubated at 37 °C for 24 h. A 1% (v/v) of the culture was inoculated into a 10 mL YPD broth, and then incubatedat 37 °C for 18 h. After incubation, the washed cell pellets were collected by centrifugation at 5,000×*g* for 10 min, and resuspended.

### 2.3. Probiotic attributes of selected yeast strains

#### *2.3.1. Growth quality at body temperature (37°C)*

The isolates was pre-cultured on YPD broth and then transferred to fresh media. Sterile YPD broths was inoculated with fresh culture of the isolate (0.1 ml) and incubated at 37 °C for 24h. Samples were taken at 0 and 24 h after incubation and the growth rate of the isolate was calculated by taking absorbance at 600 nm with a spectrophotometer [21].

$$\text{Survival \%} = \text{OD(Finial)} \div \text{OD (Initial)} \times 100$$

#### *2.3.2. Acid tolerance*

Acid tolerance was evaluated using the method described by Ogunremi et al. [14] with slight modifications. The pH of sterile YPD broth was adjusted to 3.0 with 3 mol $l^{-1}$ HCl. The broth inoculated with freshculture of the isolates and incubated at 37°C for 24 h. The samples were collected at 0 and 24 h and the tolerance rate of the strains was determined by taking absorbance at 600 nm using a spectrophotometer. Rate of survival is calculated as the percentage OD after exposure to low pH.

$$\text{Survival \%} = \text{OD(Finial)} \div \text{OD (Initial)} \times 100$$

#### *2.3.3. Bile salt tolerance*

Survival in bile salts was conducted by determining the optical density of the isolates in the YPD broth containing 1.5% of bile salts after incubation at 37°C for 24h [22]. The samples were collected at 0 and 24 h after incubation and the level of survival of the isolate determined by taking absorbance at 600 nm using a spectrophotometer.

Percentage survival is calculated with the formula:

$$\text{Survival \%} = \text{OD(Finial)} \div \text{OD (Initial)} \times 100$$

*2.3.4. Salinity (NaCl) Survival assay*

The yeast isolates were tested for their tolerance to two NaCl concentrations. The growth level of freshly cultured isolates (0.1ml) in YPD medium containing two percentages (1, 4%) of NaCl was determined via spectrophotometer at 600nm [23].

$$\text{Survival \%} = \text{OD(Finial)} \div \text{OD (Initial)} \times 100 \div 1$$

*2.3.5. In vitro Survival in gastric juice*

A slightly adapted version of the method described by Ogunremi et al. [14] was used. The washed cell pellets (1ml) was suspended in 10 ml of a solution containing simulated gastric juice (per litre, pH 2.5; 5 g NaCl and 3 g pepsin) and incubated at 37°C with agitation for 3 h. The viability of the isolate was measured by taking absorbance at 600 nm using a spectrophotometer. The level of survival was measured as the percentage OD at 3h after the simulated gastric phase and percentage survival was calculated as:

$$\text{Survival \%} = A_0 - A_1 \div A_0 \times 100$$

$A_0$ – Control $A_1$ – Sample

*2.3.6. Auto-aggregation*

Cell pellets of selected isolates obtained by centrifugation (5,000×g, 5min) were put in a 10 ml phosphate buffered saline (PBS) (0.1 mol $l^{-1}$; pH 7.0). The suspension was vortexed for 10s and incubated for 24 h at 37°C. Samples were collected carefully at 0 and 4 h and the optical density (OD) measured at 600 nm [21]. The auto-aggregation percentage was calculated as;

$$\text{Auto-aggregation (\%)} = 1 - A_t / A_0 \times 100$$

$A_t$ represents the OD at time t = 4 or 24 h; $A_0$ represents the OD at t = 0 h.

*2.3.7. Cell surface Hydrophobicity*

The cells hydrophobicity was analyzed using the method described by Diguta et al. [24] with slight modification.The yeast cells were harvested at 7000 rpm, 4ºc with a centrifuge for 10 min, washed twice and suspended in 5 ml PBS (pH 7). The optical density was measured at 600 nm and considered asA0. Aliquots (3 mL) of the cell suspensions were added to 1 mL of each hydrocarbon (chloroform and toulene) and vortexed for 120 sec. Then the suspension was kept uninterrupted at 37ºC for 30 min to allow phase separation, and the hydrocarbon layer is permitted to completely rise. After 30 min, the aqueous phase was carefully removed and the absorbance determined with a spectrophotometer at 600 nm. The decline in the absorbance is seen as a measure of the cell surface hydrophobicity (% Hydrophobicity), calculated with the equation given below:

$$H\ (\%) = OD_{initial} - OD_{final} \div OD_{initial} \times 100$$

Where, $OD_{initial}$= OD of cell pellet suspension before hydrocarbons.

$OD_{final}$= OD of aqueous phase after extraction.

*2.3.8. Susceptibility to Antibiotics*

Yeast strains obtained were tested against 10 antibacterial antibiotics with different mechanism of actions such as, ciprofloxacin, amoxycillin, erythromycin, gentamicin, ampiclox, rocephin, septrin, pefloxacin, zinacef, streptomycin, with standard antibiotic concentration measured by testing against a known pathogen. The diameter of the zone of inhibition was evaluated after 48 h of incubation at 30 °C.

**2.4. Identification of yeast isolates**

The yeast strains were cultured on a clean YPD agar media until pure cultures were obtained. Microscopic observations were conducted on wet mounted cultures using lacto phenol Blue. The morphological traits of each yeast colony were observed and noted.

### 2.5. Statistical analysis

The results obtained were statistically analyzed by means of analysis of variance (ANOVA) in the Statistical Package for Social Sciences (SPSS) and the mean differences determined by Duncan's tests at a significance level ($P < 0.05$).

## 3. Results

A total of 10 yeast strains were obtained from 2 retail sources of a Nigerian locally made cereal-based fermented food product known as Ogi (Akamu). Five (5) of the strains survived growth at 37 °C and was used throughout the study.

### 3.1. Growth survival at body temperature (37 °C)

The yeast strains were tested for their capacity to grow at internal body temperature and the selected isolates when compared to the growth at 30 °C grew favorably at 37 °C as indicated by the changes in optical density observed after 24 h of incubation (Table 1). A1 had the highest capacity to survive at 37 °C with a value of $0.584^{d}$±0.0021 which decreased down the column with the lowest at $0.455^{a}$±0.0028 for A5.

**Table 1** Growth properties of the yeasts strains at 37 °C

| Isolates | Growth at 37 °C | Growth at 30 °C |
|---|---|---|
| A1 | $0.584^{d}$±0.0021 | $0.336^{a}$±0.0035 |
| A2 | $0.538^{c}$±0.0042 | $0.429^{d}$±0.0035 |
| A3 | $0.525^{b}$±0.0028 | $0.392^{b}$±0.0028 |
| A4 | $0.523^{b}$±0.0028 | $0.406^{c}$±0.0050 |
| A5 | $0.455^{a}$±0.0028 | $0.399^{bc}$±0.0064 |

Growth rate (means ± SD) from duplicate experiments. Values without common superscript differ significantly at $P < 0.05$ (Duncan's Multiple Range Test).

**Table 2** Tolerance level of the yeasts strains to acid at pH 3

| Isolates | Acid tolerance at pH 3 | Survival (%) |
|---|---|---|
| A1 | $0.094^{bc}$±0.0028 | 61.84 |
| A2 | $0.085^{b}$±0.0035 | 81.73 |
| A3 | $0.095^{c}$±0.0035 | 82.61 |
| A4 | $0.116^{d}$±0.0050 | 62.03 |
| A5 | $0.069^{a}$±0.0035 | 60.00 |

Acid tolerance (means ± SD) from duplicate experiments. Values without common superscript differ significantly at $P < 0.05$ (Duncan's Multiple Range Test).

### 3.2. Tolerance to Low pH and Bile and Yeast's Viability

Acid and bile salt tolerance is important in the selection of a probiotic strain and is an indication of the possibility of the strain to withstand the gastric environment. The yeast strains were evaluated for their capacity to withstand low acid and bile (Table 2). All strains were able to survive at low acid at pH 3 with 1.5 % bile salts for 24 hours showing the strain's capability to favorably adjust to acid-stressed environment. For pH, highest survival of 82.61% was recorded by A3, andA5 was the most sensitive (60%), while A1 and A4 survived and maintained viability at 61.84 and 62.03% respectively. All of the yeast strains showed ability to survive a bile concentration of 1.5% after 24 h incubation (Table 3). A3 was highly tolerant, maintaining 83.67% viability, with A2 giving the lowest tolerance at 62.96%. These results indicate their ability to survive an acid/bile environment of the gut.

### 3.3. Sodium Chloride tolerance

The yeast strains performed well at high salt content as shown in fig. 1. At a salt level of 1%, A4 and A5 had the highest tolerance with a percentage of 89.29 and 87.10 respectively. When the salt concentration was increased to 4%, A4

experienced an increase to 92.86% while A5 decreased by 4%. A1 and A2 also experienced an increase from 77.43 – 83.87% and 81.48 – 85.19% respectively but A4 was more tolerant to salt than the rest.

### 3.4. Survival atin vitro gastric juice

The yeast strains were estimated for their survival rate in gastric environment using stimulated gastric juice. The survival of A2 reduced to 42.86% after 3 h incubation, having the highest ability to survive the stimulated gastric juice (Fig 2). No survival was recorded for A1, while A4 gave the lowest percentage survival of 7.69%.

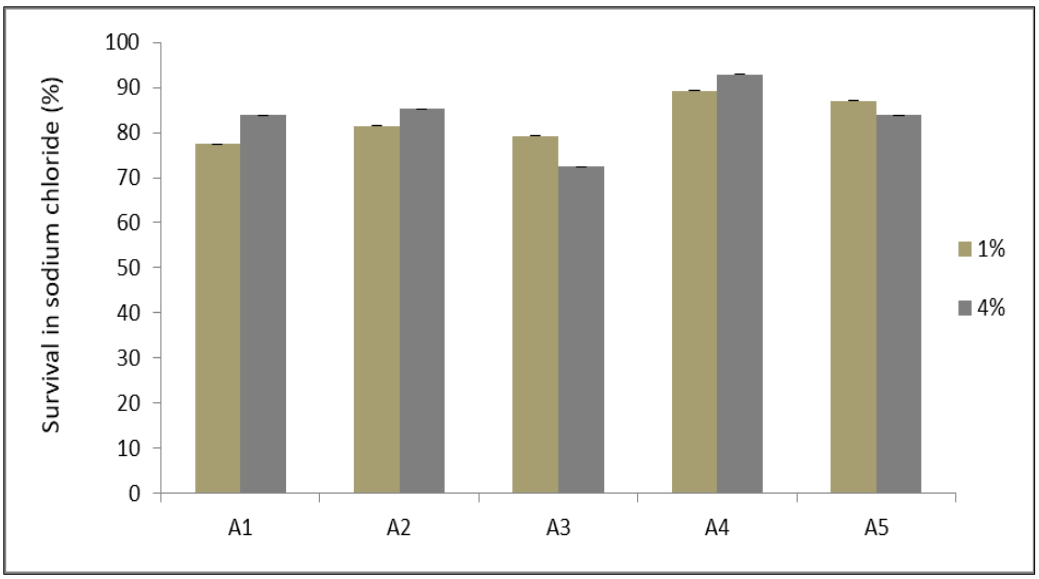
### 3.5. Auto-aggregation

The auto-aggregation capacities of the yeast strains were evaluated, and all were above 60% after an incubation period of 24 h. A4 had the highest percentage auto-aggregation (94.9%) while A3 has the lowest with 68.8% as shown in Fig. 3.

**Table 3** Tolerance level of the yeasts strain to bile salt

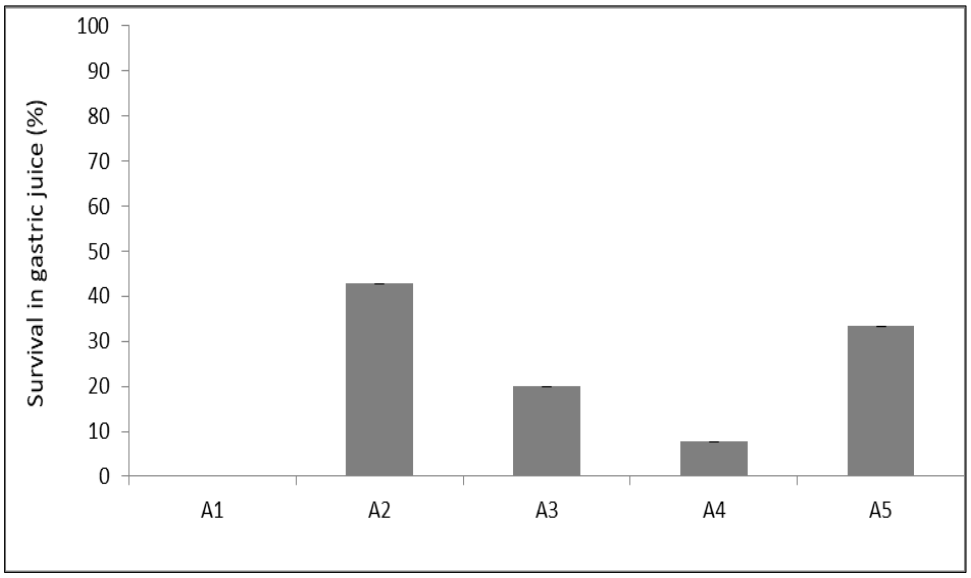
| Isolates | Bile salt tolerance (1.5%) | Survival (%) |
|---|---|---|
| A1 | $0.041^{a}$±0.0035 | 74.54 |
| A2 | $0.034^{a}$±0.0028 | 62.96 |
| A3 | $0.041^{a}$±0.0064 | 83.67 |
| A4 | $0.040^{a}$±0.0014 | 78.43 |
| A5 | $0.040^{a}$±0.0042 | 70.18 |

Bile salt tolerance (means ± SD) from duplicate experiments. Values without common superscript differ significantly at P < 0.05 (Duncan's Multiple Range Test).




Sodium chloride (means ± SD) from duplicate experimentsat P < 0.05 (Duncan's Multiple Range Test).

**Figure 1** Tolerance level of the yeasts strain to sodium chloride




Tolerance to gastric juice (means ± SD) from duplicate experimentsat P < 0.05 (Duncan's Multiple Range Test).

**Figure 2** Tolerance level of the yeast strain to stimulated gastric juice

### 3.6. Hydrophobicity

The surface hydrophobicity of the yeast strains was determined by checking the level of adhesion of the yeast strains to chloroform and toluene as shown in fig. 4. The hydrophobicity ranged between 41.2-61.5% for chloroform and in toluene, ranging between 14.3-36.4%, respectively. A4 showed the highest hydrophobicity towards chloroform with 61.5% and was lower (26.1%) for toluene. The highest hydrophobicity for toluene was A5 with 36.4%.

### 3.7. Antibiotics susceptibility

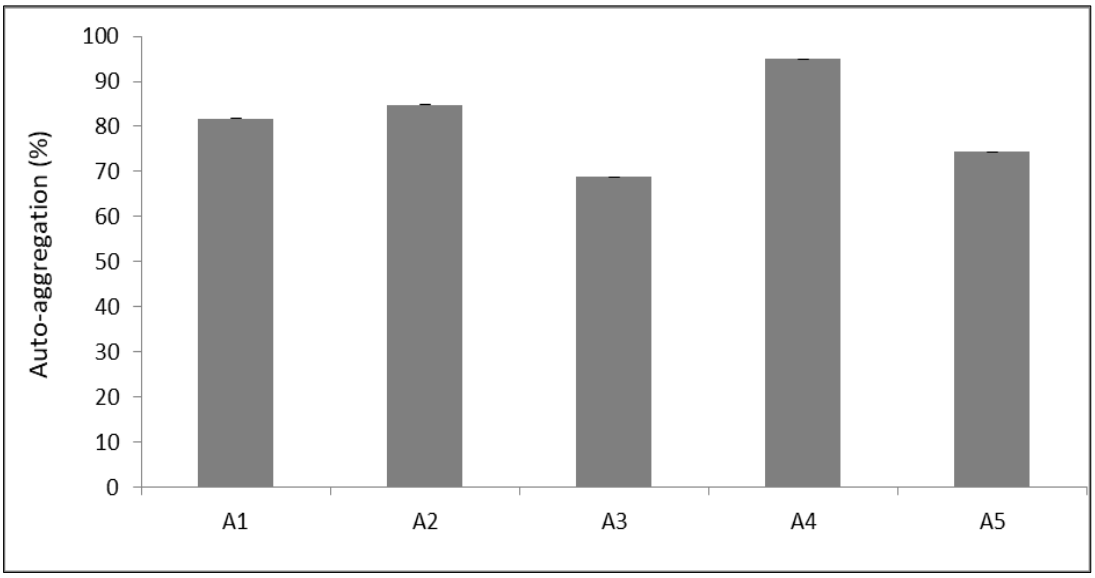
Antibiotics susceptibility profile of the yeasts to 10 antibiotics was analyzed (Table 4). Antibiotic susceptibility test showed that all the tested yeasts strains were resistant to every one of the antibacterial antibiotics.

### 3.8. Identification of yeast strains

The five different yeast strains based on their morphology and microscopic characteristics were identified as *Saccharomyces cerevisiae* (A1, A2, A3, A5) and *Candida tropicalis* (A4).

## 4. Discussion

Yeasts have shown to be very important in biotechnological applications as starter cultures in the production of beneficial high value functional foods as a result of its enhanced fermentative potential [25]. This has sparked great interest in characterization of new and improved probiotic yeasts, since yeast probiotic are so far limited to *S. cerevisae* and *S. boulardii* which have been clinically certified for use in humans with positive health influence on the host through nutritional effect, antimicrobial activities, inactivation of bacterial toxins, anti-inflammatory activities, immuno-modulatory outcomes, maintenance of epithelial barrier integrity and cell restitution [26, 27]. The present study aimed to assess the *in vitro* probiotic capability of five yeast strains obtained from locally produced cereal-based fermented food. A probable probiotic must have the ability to grow optimally and function at the body temperature (37 °C). The yeast strains tested showed strong capability to grow at 37 °C. This was also observed by Ogunremi et al. [14] where all the yeast cells obtained from cereal based fermented foods grew well at 37 °C. Resistance to acid and bile are potential indicator of a probiotic strain to withstand the acidic conditions of the stomach (pH 2.5-3.0).




Auto aggregation (means ± SD) from duplicate experiments at P < 0.05 (Duncan's Multiple Range Test)

**Figure 3** Auto-aggregation of the yeast strains

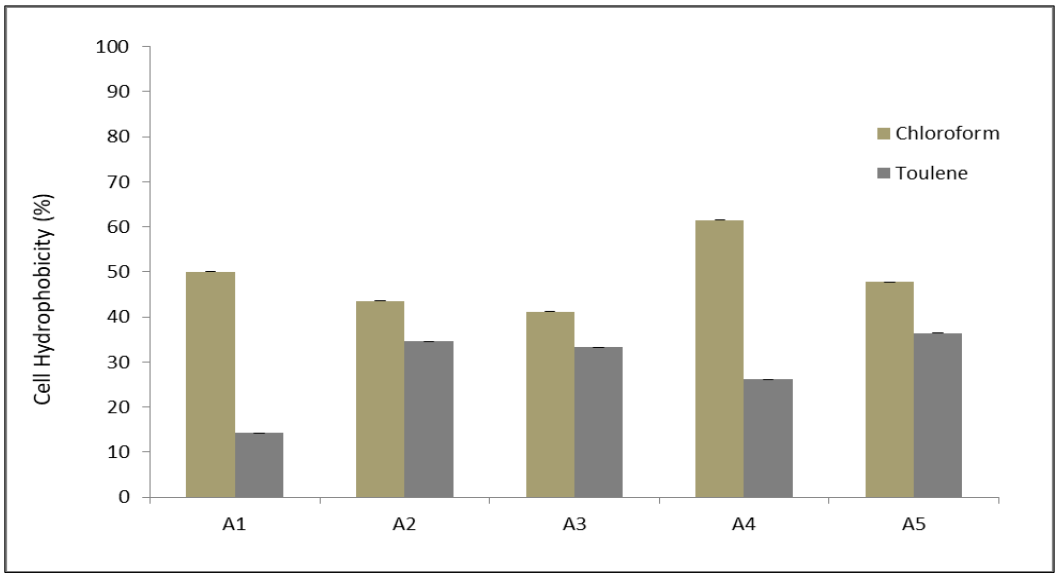
The yeast strains in our study showed high acid tolerance at pH 3 with highest recorded for A3 with 82.61% survival. Similar results were gotten by Diguta et al. [24], where all the yeast strains displayed high tolerance to the low pH, from 1.5–3.5. The high percentage survival demonstrated within 24 h incubation is indication of their ability to survive long exposures

Bile tolerance is very important due to their antibacterial activities and a concentration of 0.3% in human is considered significant in the selection of probiotics.

All the yeast strains in this research displayed tolerance to 1.5% bile concentration with the percentage survival ranging from 62.96 to 83.67%. Diguta et al. [24] reported high tolerance to bile salt of 0.3% after 4h, also Ogunremi et al. [14] reported a tolerance of up to 2% bile salt concentration. High tolerance to bile is important to determine the persistence of a potential probiotics in the gastrointestinal tract.

Probiotic yeast has several advantages over the bacteria like their tolerance to salt, since the salt stress is capable of affecting bacteria survival rate. The yeast strains in this study grew well under high salt concentrations (1 and 4%) with a tolerance level ranging between 77.4 - 89.3% and 72.4 – 92.9% respectively across the yeast strains. This was also observed by Fakruddin et al. [27] showing growth and tolerance of *S. cerevisiae* IFST062013 to NaCl with a sharp decline in growth up to 8% salt concentration.

After exposure to stimulated gastric conditions, the yeasts demonstrated ability to survive passage via the gastrointestinal tract with varying percentage survival. Auto-aggregation and cell surface hydrophobicity is a major factor in the colonization of microorganisms in the gut due to the organism's ability to adhere to the epithelial cells and is a central property of a potential probiotics [28, 27]. Auto-aggregation of the yeast strains in the current study revealed very high percentage ability (68.8-94.9%). Syal and Vohra [29] reported auto-aggregation ability higher than 95% for all isolates after 20 h. Hydrophobicity to chloroform and toluene was observed and the yeast showed variable hydrophobicity affinity. This is in agreement with research previously reported [21, 24]. The safety property of the yeast strains was evaluated and all gave resistance to the antibacterial antibiotics making them appropriate for use in treatment involving bacteria pathogens. The results gotten from this study are encouraging reference to the potential of yeast (*S. cerevisiae* and *C. tropicalis*) as probiotics through in vitro trials. Follow-up studies are required for in vivo analysis, to determine the safety propertiesof *C. tropicalis,* since *S. cerevisiae* has been clinically proven for use in human.




Hydrophobicity (means ± SD) from duplicate experiments at P < 0.05 (Duncan's Multiple Range Test).

**Figure 4** Cell hydrophobicity of the yeasts strains

**Table 4** Antibiotics susceptibility of the yeast strains

| YEAST STRAINS | AM 10 µg | CIP 10 µg | ERY10 µg | GEN 10 µg | AMP 30 µg | ROC 25 µg | SEP 30 µg | PEF 10 µg | ZIN 20 µg | STRE 30 µg |
|---|---|---|---|---|---|---|---|---|---|---|
| A1 | R | R | R | R | R | R | R | R | R | R |
| A2 | R | R | R | R | R | R | R | R | R | R |
| A3 | R | R | R | R | R | R | R | R | R | R |
| A4 | R | R | R | R | R | R | R | R | R | R |
| A5 | R | R | R | R | R | R | R | R | R | R |

**Legend**: R-Resistant, S-Sensitive, AM (Amoxycillin), CIP (Ciprofloxacin), ERY (Erythromycin), GEN (Gentamicin), AMP (Ampiclox), ROC (Rocephin), SEP (Septrin), PEF (Pefloxacin), ZIN (Zinacef), STRE (Streptomycin).

## 5. Conclusion

We successfully isolated yeast strains with probiotic capability from African cereal-based fermented foods. Since Ogi is a popularly cereal made and consumed by the local communities, it is vital to note that the fermenting cultures offer a wide varieties of health advantages and can be used as a vehicle for the delivery of probiotics. Yeasts have great potential for applications in both animal and human food formulations which can be done through additional examinations to ensure safety and efficiency. *S. cerevisiae* and *C. tropicalis* have shown several beneficial properties that make them potential probiotic candidates and can be used for future applications.

## Compliance with ethical standards

### *Acknowledgement*

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### *Disclosure of conflict of interest*

The authors state that there are no conflicts of interest in the publication of this article.

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