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# Antagonistic pattern of yeast species against some selected food-borne pathogens

Ilesanmi Festus Fadahunsi\* and Simbo Olubodun

## Abstract

**Background:** The efficiency of synthetic preservation in shelf life extension of food is well documented, but the hazardous side effects associated with it are posing serious threat to the food industry. Therefore, this present research work is aimed at determining the suitability of yeast metabolite as an alternative natural preservative agent of food.

**Results:** The results obtained revealed that seventy-three yeast isolates were obtained from the fruits and were identified as *Candida pelliculosa*, *Kluyveromyces phaffii*, *Metschnikowia pulcherrima*, *Saccharomyces cerevisiae* 001, *Saccharomyces cerevisiae* 002, *Saccharomyces cerevisiae* 003 and *Rhodotorula mucilaginosa* and were confirmed safe. The highest production of lactic acid (3.6) and diacetyl (1.0 mg/ml) was recorded by *Saccharomyces cerevisiae* 001 and the best pH, temperature, sodium chloride concentration, carbon and nitrogen sources that stimulated maximum inhibitory activities of the yeast species against the food-borne pathogens were 4, 30 °C, 2–4%, glucose and skimmed milk, respectively. The lowest MIC and MBC values recorded were  $3.125 \pm 0.6$  and  $6.25 \pm 0.26.25 \pm 0.3$  µg /ml, respectively.

**Conclusion:** The study confirmed that the combination of the yeast metabolites could be potentially used as bio-preservation in food.

**Keywords:** Antagonistic pattern, Yeast species, Food-borne pathogens, Yeast metabolites, Zone of inhibition

## Background

Yeast species are vividly grouped into the fungal kingdom based on the possession of a chitinous well-defined cell wall. They are unicellular eukaryotic fungi devoid of peptidoglycan with lipid-linked ester. Morphologically, they vary from round to ovoid, 5–10 µm in diameter and exhibit both asexual and asexual modes of reproduction (Kurtzman et al. 2010, 2011). They are isolated from sugar-rich environments, skins of fruits, fermented foods and exudates of plants (Slavikova et al. 2007). In addition, they are resident in humans toe as part of skin flora, gut flora of mammals, some insects and deep sea environment, but the ecological function and biodiversity of yeast species are relatively unknown compared to other micro-organisms (Kutty et al. 2008; Herrera et al. 2010). There are documented reports on their survival in both

aerobic and anaerobic environments (Balasubramanian 2004) and are known biochemically as non-lactose and cellobiose fermenters. They have been reported to survive a temperature range of 5–35 °C and freezing under certain conditions with decreasing viability per time (Arthur and Watson 1976). Yeast can either exist in free state or constitute synergy such as parasitism, symbiosis, mutualism and competition with other micro-organisms (Goldman 2008). Nutritionally, their complete growth requirement is simple, which comprises carbon, a nitrogen source (ammonium salt, nitrate, amino acid, peptide, urea, purine and pyrimidine), phosphate, sulphate, trace amount of potassium, magnesium, calcium, iron, zinc and a vitamin source such as biotin, thiamine, pantothenic acid (Deak 2006). It had been reported that yeast species secrete some inhibitory compounds such as diacetyl, organic acids, mycocins, antibiotic factors, volatile acids and various other products, which are capable of eliciting inhibition against food-borne pathogens and food spoilage micro-organisms, but the mechanism of

\*Correspondence: sanmifadahunsi@yahoo.com  
Department of Microbiology, Faculty of Science, University of Ibadan,  
Ibadan, Oyo State, Nigeria

inhibition is unknown (Oyewole 1997; Viljoen 2006). According to Druvefors et al. (2005), food fermentations are naturally carried out by an interacting complex microbial population comprising of bacteria, mould and yeast. It is important to note that reports on the production of allergy-causing spores and mycotoxins by yeast are unavailable, but the synthesis of antimicrobial metabolites is well documented, and these special attributes accord them their acceptability as biopreservative agent and starter cultures in food fermentation (Druvefors et al. 2005). Similarly, Hara et al. (1980), Pfeiffer and Radler (1984), Seki et al. (1985), Boone et al. (1990), Van Vuuren and Jacobs (1992), Comitini et al. (2004a, b) reported that genetically improved antagonistic yeast starter cultures contribute to improved safety of food, sensory qualities and shelf life of the finished product when inoculated into the fermenting medium by inhibiting the growth of associated pathogenic bacteria. According to Young (1987) and Boone et al. (1990), starter yeasts inhibit wild and undesirable yeast strains during beer and bread production. In addition, they also inhibit the growth of undesirable yeast during food preservation (Palpacelli et al. 1991) and as a therapeutic agent (Cailliez et al. 1994). From past research findings, it is observed that most of its applications are based on exploiting its antagonistic capabilities (Zhao et al. 2020). Summarily, it is used in food and agriculture for producing microbiologically stable fermented food products with good organoleptic properties, bio-control agents for soil treatment and prevention of pre- and post-harvest diseases of crops (Pretorius 2000). In addition, *Candida oleophila* has been registered as a standard bio-control agent for post-harvest crop diseases (Entlaeshawy and Wilson 1997; Santos and Marquina 2004). It could be suggested that antagonistic yeasts could be a potential alternative bio-preservative to conventional chemical preservatives, which presently constitute threats to human lives (Chiquette 2009). There is an increasing interest in utilizing probiotic micro-organisms for treating gastrointestinal tract (GIT) disorders and yeast strains acting as antagonists toward spoilage or pathogenic microbes (O'sullivan et al. 2010; Maccaferri et al. 2012; Fernández et al. 2015). In addition, probiotic yeasts have shown inhibitory activity against pathogenic bacteria. Kumura et al. (2014), Psani and Kotzekidou (2006), Klingberg et al. (2005), Generoso et al. (2010) had reported the wide use of yeast species in probiotic preparations, and several studies have confirmed the positive interaction of yeast with probiotic bacteria by enhancing their survival and stimulating their growth (Hodgson et al. 1995; Katakura et al. 2010; Suharaja et al. 2012). Kelsesidis and Pothoulakis (2012) had reported the application of yeast metabolites in treatment of diarrhoea and candidiasis. Furthermore, Kurtzman et al. (2011)

confirmed the effectiveness of yeast single cell protein in accelerating growth and conferring health benefits to cattle when fed into them.

Therefore, this present study is aimed at providing additional information on the antimicrobial property of yeast species against food-borne pathogens, which constitute serious public health problems in both developed and developing nations.

## Methods

### Sample collection

Apple, oranges, mango, pawpaw, watermelon, cashew and pineapple were purchased from various locations within Ibadan, Oyo state, Nigeria, in May 2020. The juice of each fruit was obtained using juice extractor and was used for isolation of yeast species in the laboratory.

### Test organisms

The test bacteria used in this research were *Salmonella* sp (S.S), *Campylobacter jejuni* (C.J), *Listeria monocytogenes* (L.M) PCM2191 and *Vibrio cholera* (V.C). The *Salmonella* sp and *Vibrio cholerae* were obtained from the culture collection of the Food Microbiology Unit, Department of Microbiology, University of Ibadan, while the *Campylobacter jejuni* was obtained from the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan. The *Listeria monocytogenes* PCM2191 was obtained from Dr I.F. Fadahunsi of the Department of Microbiology, University of Ibadan. The indicator bacteria were subcultured repeatedly to obtain pure cultures, which were stored on agar slants in McCartney bottles and kept in a refrigerator at 4 °C.

### Isolation of yeast species from the food items

This was carried out by weighing 10 g of the juice of each sample into 90 ml of sterilized distilled water in 150-ml Erlenmeyer flasks, and serial dilution was carried out according to the method described by Meynell and Meynell (1965). The malt extract agar plate containing 50 µg/ml Streptomycin was inoculated with 0.1 ml from the 10<sup>-5</sup> dilution test tube of the sample using the spread plate method and incubated at 30 °C for 72 h. The plates were examined for microbial growth, and representative colonies were selected and subcultured repeatedly, until pure cultures were obtained and stored on malt extract agar slant in McCartney bottles and kept at 4 °C in a refrigerator. This treatment was repeated for the other fruit samples.

### Pathogenicity test

#### *Gelatin liquefaction*

One ml from 24-h-old culture of the yeast cells suspension was inoculated into sterile gelatin medium in 100-ml Erlenmeyer flasks containing 10% malt extract and incubated at 30 °C for 7 days; the temperature was reduced to observe liquefaction. At lower temperature, liquefaction of gelatin indicates positive reaction, while un-liquefied gelatin indicates negative reaction (Walt and Yarrow 1984).

### Haemolysis test

A colony from 24-h-old culture of the yeast cells was streaked on blood agar plates and incubated for 24–72 h at 30 °C and were observed for alpha, beta or gamma haemolytic reaction (Olutiola et al. 1991).

### DNASE test

The method described by Olutiola et al. (1991) was adopted by picking a colony from a 24-h-old culture of yeast cells and streaked on DNase agar plates containing methyl green indicator and incubated at 37 °C for 72 h. The plate was observed for green colour fades surrounded by a colourless zone.

### Characterization of yeast isolates from food items

All the isolates were characterized based on their ability to assimilate carbon, nitrogen compounds and subjecting them to physiological tests as described by Sanni and Lonner (1993).

### Preparation of cell-free metabolites of the isolated yeast species

One ml ( $1.0 \times 10^7$  cfu/ml) of a 48-h-old yeast cells suspension was inoculated into 100 ml of sterilized yeast extract peptone dextrose broth (YEPD) in 150-ml Erlenmeyer flask and incubated for 72 h. Centrifugation was carried out for 15 min at 2800 rpm to obtain the supernatant, which was used to determine the antimicrobial activity of the yeast species.

### Quantitative measurement of inhibitory compounds produced by the yeast species

#### *Lactic acid*

Three drops of phenolphthalein indicator were added to 25 ml of each supernatant, in 150-ml Erlenmeyer flask, and titrated against 0.1 M sodium hydroxide until a pink colour appeared. One ml of 0.1 M sodium

hydroxide solution is equivalent to 90.80 g of lactic acid (AOAC 2003).

### *Diacetyl*

This was done by transferring 25 ml of the supernatant into 150-ml Erlenmeyer flask containing 7.5 ml of hydroxylamine solution, and 0.1 M hydrochloric acid (HCl) was gradually added to the flask until greenish-yellow end point was reached using bromophenol blue as an indicator. The equivalent factor of HCl to diacetyl is 21.5 mg (AOAC 2003).

### Preparation of inoculums

A 24-h-old bacterial cell suspension of the indicator organisms was differently transferred into 5 ml of normal saline, and the density of the inoculums was adjusted to 0.5 McFarland turbidity standards, resulting in a suspension of  $1.0 \times 10^7$  cfu/ml of each of the test bacteria (Bhalodia and Shukla 2011).

### Determination of antagonistic activity of the cell free metabolite of the yeast species

Mueller–Hinton agar was poured aseptically into the plates, which were previously seeded with the different standardized test bacteria, and wells were bored aseptically in the agar using 6-mm cork borer and filled with the different yeast metabolite. The plates were allowed to stay at 30 °C for 30 min to allow diffusion of the yeast metabolite into the agar and incubated at 30 °C for 24 h. Zones of inhibition around the wells were measured in millimetre.

### Preparation of basal medium used for supplementation study

The basal medium used for this study consisted of magnesium sulphate hepta-hydrate  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.05 g), potassium dihydrogen phosphate  $\text{KH}_2\text{PO}_4$  (0.05 g), potassium nitrate (1.5 g) in 1 L sterile distilled water in 2-L Erlenmeyer flask.

### Effect of pH on the antimicrobial activity of the cell-free metabolite of the yeast species

Twenty mls of the yeast extract peptone dextrose medium (YEPD) at pH 3, 4, 6 and 7 was dispensed into several 50-ml Erlenmeyer flasks and sterilized at 121 °C for 15 min and inoculated with one ml ( $1.0 \times 10^7$  cfu/ml) of a 48-h-old cells suspension of yeast species. Incubation and centrifugation were carried out as described earlier, and antimicrobial property was determined using the cell-free metabolite as described earlier (Singh et al. 2012).

#### **Effect of temperature on the antimicrobial activity of cell-free metabolite of the yeast species**

Twenty mls of the yeast extract peptone dextrose medium (YEPD) was dispensed into several 50-ml Erlenmeyer flasks, sterilized at 121 °C for 15 min and inoculated with one ml ( $1.0 \times 10^7$  cfu/ml) of a 48-h-old cells suspension of the yeast species. Incubation and centrifugation were carried out as described earlier, and the supernatant obtained was dispensed in equal amount into various test tubes and differently incubated at 30 °C, 35 °C, 40 °C and 45 °C. The antimicrobial activity was determined as described earlier (Singh et al. 2012).

#### **Effect of sodium chloride concentration on the antimicrobial activity of the cell-free metabolite of the yeast species**

Twenty mls of the basal medium was dispensed into several 50-ml Erlenmeyer flasks, and 2%, 4% and 6% NaCl (w/v) were differently added. Sterilization was carried out at 121 °C for 15 min and inoculated with one ml ( $1.0 \times 10^7$  cfu/ml) of a 48-h-old cells suspension of the yeast species. Incubation and centrifugation were carried out as described earlier, and the supernatant obtained was used to determine the antimicrobial activity as described earlier (Singh et al. 2012). The flask containing sterile basal medium without sodium chloride was inoculated with one ml ( $1.0 \times 10^7$  cfu/ml) of a 48-h-old cells suspension of the yeast species and served as the control.

#### **Effect of carbon source on the antimicrobial activity of cell-free metabolite of the yeast species**

The carbon sources used in this study consisted of glucose, fructose, xylose, galactose, lactose, sucrose, maltose, raffinose, sorbitol and mannitol. Fifty mls of the basal medium was dispensed into several 100-ml Erlenmeyer flasks sterilized at 121 °C for 15 min and dispensed in equal amount into various test tubes. The carbon sources used were sterilized at 110 °C for 10 min and separately aseptically added at 0.8% (w/v) concentration to the basal medium in different test tubes and inoculated with one ml ( $1.0 \times 10^7$  cfu/ml) of a 48-h-old cells suspension of the yeast species. Incubation and centrifugation were carried out as described earlier, and the supernatant obtained was used to determine the antimicrobial activity as described earlier (Singh et al. 2012). The flask containing sterile basal medium with no carbon source was inoculated with one ml ( $1.0 \times 10^7$  cfu/ml) of a 48-h-old cells suspension of the yeast species and served as the control.

#### **Effect of nitrogen sources on the antimicrobial activity of the cell-free metabolite of the yeast species**

The nitrogen sources used in this study included alanine, aspartic acid, asparagine, tryptophan (simple nitrogen sources), peptone and skimmed milk (complex nitrogen sources). The simple nitrogen sources were supplemented at 0.1%, while the complex nitrogen sources were supplemented at 1% concentration into the growth medium (Oso 1974). Inoculation, incubation, centrifugation and antimicrobial property were done as reported earlier. The flasks containing basal medium without nitrogen source were inoculated with one ml ( $1.0 \times 10^7$  cfu/ml) of a 48-h-old cells suspension of the yeast species and served as the control.

#### **Determination of minimum inhibitory concentration of supernatant of the yeast species**

The minimum inhibitory concentration (MIC) of the cell-free metabolite of the yeast species was estimated for each of the tested bacteria in triplicate using the broth dilution method described by Singh et al. (2012). Tubes containing double-fold dilution of cell-free metabolite of the yeast species in 1 ml of nutrient broth were inoculated with 1 ml ( $1.0 \times 10^7$  cfu/ml) of the standardized test bacteria, and a tube containing nutrient broth only was seeded with the test bacteria, which served as control. Incubation was carried out at 37 °C for 24 h, and the tubes were examined for bacterial growth by viewing turbidity and the test tube that appeared clear/not turbid (no microbial growth) was considered as the MIC. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the metabolite that showed no visible growth (no turbidity) when compared with the control tube.

#### **Determination of minimum bactericidal concentration of cell-free supernatant of the yeast species**

The minimum bactericidal concentration (MBC) was determined by streaking from each of the MIC (non-turbid) test tubes on nutrient agar and incubated at 37 °C for 24 h. The lowest concentration of the extract at which the bacteria were killed was regarded as MBC. Each experiment was done in duplicate.

## **Results**

The result of the frequency of occurrence of the yeast isolates is shown in Table 1. It was observed that a total of seventy-three of the yeast isolates were obtained from the juice of the fruits, and the distribution is shown in Table 1

**Table 1 Frequency of occurrence**

Isolates	Frequency of occurrence
AJ4 Isolate from apple juice	10
MK4 Isolate obtained from mango juice	5
PA2 Isolate obtained from pineapple juice	17
PW1 Isolate obtained from water melon juice	9
PW4 Isolate obtained from pawpaw juice	12
OR1 Isolate obtained from cashew juice	5
OR2 Isolate obtained from orange juice	15
Total no. of isolates	73

**Table 2 Pathogenicity test of yeast isolates**

Isolate code	Gelatin liquefaction	Haemolytic test	Dnase test
AJ4	-	-	-
MK4	-	-	-
PA2	-	-	-
PW1	-	-	-
PW4	-	-	-
OR1	-	-	-
OR2	-	-	-

-, No activity

The result of the pathogenicity tests conducted on the yeast isolate is shown in Table 2. It can be inferred from the result that all the yeast species tested negative to gelatin liquefaction, haemolytic and DNase tests.

The result of the microscopic morphological appearance of the yeast species shows the shape, colour, elevation, edge, texture, budding cells and appearance of pseudo-hyphae in Table 3. Isolate AJ4 Looks like a creamy flat smooth colony, showing a butyrous texture with single budding. Isolate MK4 was seen under the microscope as a raised ellipsoidal structure with serrated edge. It is mucoid, creamy in colour showing multiple budding with pronounced pseudo-hyphae. Isolate PA2 is a spherically flat structure with smooth edge. It is butyrous and creamy in colour showing single-polar budding. Isolate PW1 was seen in under the microscope as spherical in shape with raised smooth edge, butyrous in nature showing creamy colour and unipolar budding. Isolate PW4 appeared as a flat serrated ellipsoidal-shaped colony, which is butyrous showing single-polar budding with creamy colouration. Isolate ORI was observed under the microscope as a smooth spherically raised creamy colony displaying butyrous texture with unipolar budding. Isolate OR2 was observed under the microscope as a smooth spherically raised pinkish colony displaying butyrous texture accompanied by single budding.

**Table 3 Microscopic morphological appearance of the yeast isolates**

Isolate code	Shape	Elevation	Edge	Texture	Colour	Lactophenol cotton blue
AJ4	Ellipsoidal	Flat	Smooth	Butyrous	Creamy	Single budding cell
MK4	Ellipsoidal	Raise	Serrated	Mucoid	Creamy	Multiple budding with pronounced pseudo-hyphae
PA2	Spherical	Flat	Smooth	Butyrous	Creamy	Single-polar budding
Pw1	Spherical	Raise	Smooth	Butyrous	Creamy	Unipolar budding
Pw4	Ellipsoidal	Flat	Serrated	Butyrous	Creamy	Singly budding cells
OR1	Spherical	Raise	Smooth	Butyrous	White	Unipolar budding cells
OR2	Spherical	Raise	Smooth	Butyrous	Pinkish	Singly budding

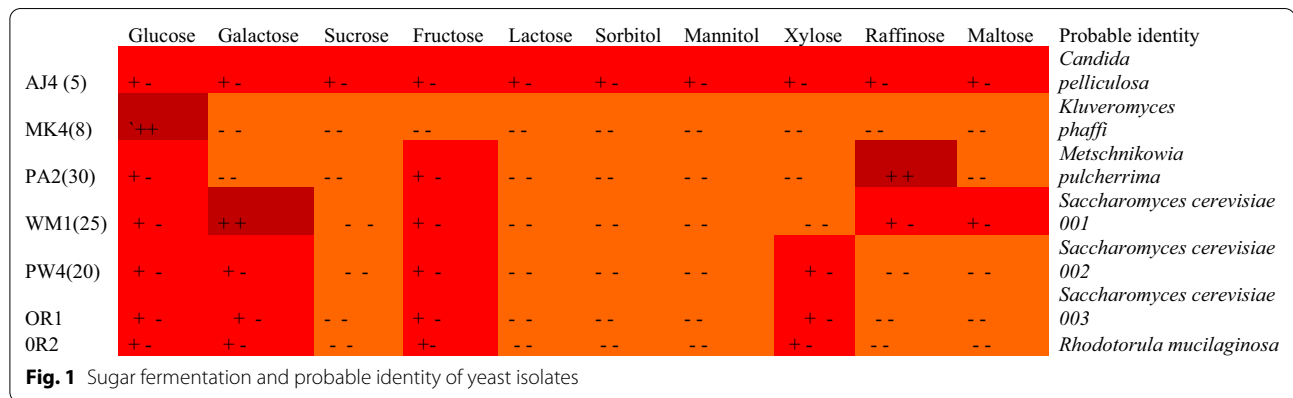




Figure 1 shows the sugar fermentation pattern of the different yeasts species used in this study. AJ4 utilized glucose, galactose, fructose and maltose but did not utilize raffinose, sucrose, lactose, sorbitol, mannitol, xylose. Isolate MK4 utilized glucose with colour change and gas production, but did not utilize galactose, sucrose, fructose, lactose, sorbitol, mannitol, xylose, maltose and raffinose, while isolate PA2 utilized glucose, fructose with colour change, but no gas production. Raffinose was utilized with colour change and gas production. However, galactose, sucrose, lactose, sorbitol, mannitol, xylose and maltose were not utilized. Isolate PW1 utilized glucose, fructose, raffinose and maltose with colour change, but no gas production. Galactose was utilized with both colour change and gas production. However, sucrose, sorbitol, mannitol, xylose and lactose were not utilized. Isolate PW4 also utilized glucose, galactose, fructose and xylose with colour change but no gas production. The isolate did not utilize sucrose, lactose, sorbitol, mannitol, raffinose and maltose.

OR1 utilized glucose, galactose, fructose and raffinose with colour change, but no gas production but did not utilize sucrose, lactose, sorbitol, mannitol, xylose and maltose.

OR2 did not utilize any of the sugars used in this study. However, isolate AJ4 was able to utilize glucose,

galactose, sucrose and maltose with colour change and gas production, but did not utilize fructose, lactose, mannitol, xylose, sorbitol and raffinose.

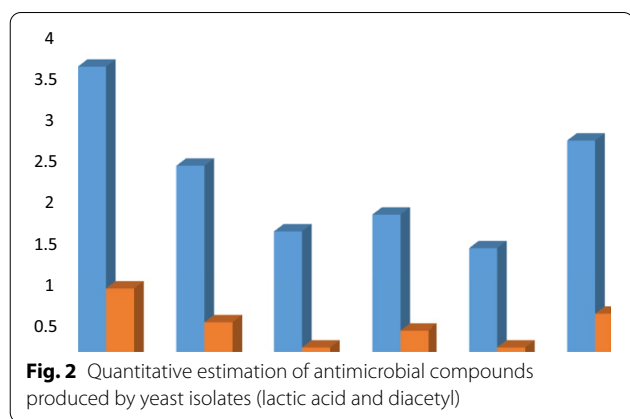
Results in Fig. 1 were combined together to probably identify isolates AJ4, Mk4, PA2, PW1, PW4, OR1 and OR2, as *Candida pelliculosa*, *kluveromyces phaffii*, *Metschnikowia pulcherrima*, *Saccharomyces cerevisiae* 001, *Saccharomyces cerevisiae* 002, *Saccharomyces cerevisiae*.003 and *Rhodotorula mucilaginosa*, respectively.

The result of quantification of lactic acid and diacetyl produced by the yeast species is presented in Fig. 2. *Saccharomyces cerevisiae* 001 produced the highest quantity of lactic acid and diacetyl of 3.6 mg/ml and 1 mg/ml, respectively, while the least quantity of lactic acid was produced by *Metschnikowia pulcherrima* and the least diacetyl was produced by *Candida pelliculosa* and *Metschnikowia pulcherrima*.

The result of the antimicrobial activity of the yeast species against the food-borne pathogens is shown in Table 4. The results obtained showed that the widest zone of inhibition of  $25.0 \pm 0.4$  mm was recorded by *Saccharomyces cerevisiae* (001) against *Campylobacter jejuni*.

Effect of different pH on the antimicrobial activities of the yeast species is represented in Fig. 3a–d. The pHs used for this study were 3, 4, 5, 6 and 7. The best pH that favoured the highest inhibitory activity of the cell-free supernatant of the yeast species was 4 at which *Saccharomyces cerevisiae* 001 showed the highest zone of 30.00.9 mm against *Campylobacter jejuni*, while it showed an inhibition a zone of  $25.0 \pm 0.4$  mm against *Campylobacter jejuni* in the control experiment. The least zone of inhibition at pH 4 was  $9.0 \pm 0.3$  mm exhibited by *Rhodotorula mucilaginosa* against *Salmonella* sp.

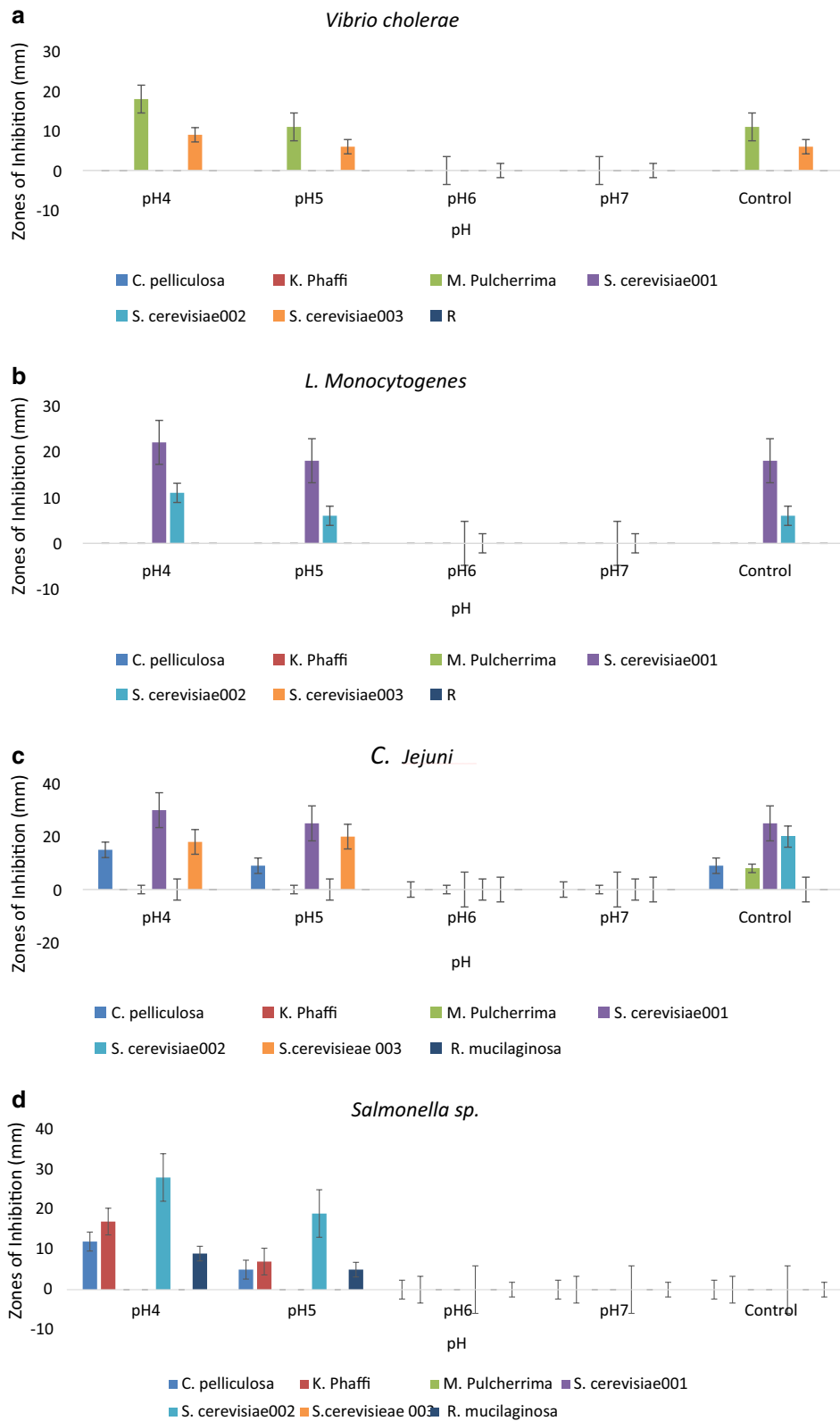
Table 5a and b represents the result of effect of different temperatures on the inhibitory activity of the yeast species. The different temperature used in this study included 30 °C, 35 °C, 40 °C and 45 °C. The result revealed that the best temperature (30 °C)



**Table 4** Zone of inhibition of yeast isolates against food-borne pathogens

Yeast isolates	<i>Vibro cholerae</i>	<i>Listeria monocytogene</i>	<i>Campylobacter jejuni</i>	<i>Salmonella</i> sp.
<i>Candida pelliculosa</i>	–	–	9.0 ± 0.02	5.0 ± 0.5
<i>Kluveromyces phaffii</i>	–	–	–	7.0 ± 0.2
<i>Metschnikowia pulcherrima</i>	11.0 ± 0.2	–	8.0 ± 0.5	–
<i>Saccharomyces cerevisiae</i> 001	–	18.0 ± 0.1	25.0 ± 0.4	–
<i>Saccharomyces cerevisiae</i> 002	–	16.0 ± 0.3	20.0	19.0 ± 0.8
<i>Saccharomyces cerevisiae</i> 003	6.0 ± 0.1	–	–	13.0 ± 0.4
<i>Rhodotorula mucilaginosa</i>	–	–	–	5.0 ± 0.2

–, No activity



**Fig. 3** **a** Effect of different pH on the antimicrobial activities of the yeast species on *Vibrio cholerae*. **b** Effect of different pH on the antimicrobial activities of the yeast species on *Listeria monocytogenes*. **c** Effect of different pH on the antimicrobial activities of the yeast species on *C. jejuni*. **d** Effect of different pH on the antimicrobial activities of the yeast species on *Salmonella*

**Table 5 Effect of temperature on the microbial inhibitory activity (mm) of the yeast species**

Yeast species	Vibrio cholera				Listeria monocytogenes			
	30 °C	35 °C	40 °C	45 °C	30 °C	35 °C	40 °C	45 °C
(a)								
<i>Candida pelliculosa</i>	–	–	–	–	–	–	–	–
<i>K. phaffii</i>	8.0	–	–	–	–	–	–	–
<i>M. pulcherrium</i>	11 ± 0.7	–	–	–	–	–	–	–
<i>S. cerevisiae</i> 001	–	–	–	–	18.0	–	–	–
<i>S. cerevisiae</i> 002	–	–	–	–	16.0	20.0	–	–
<i>S. cerevisiae</i> 003	6.0 ± 0.3	–	–	–	–	–	–	–
<i>R. mucilaginosa</i>	–	–	–	–	–	–	–	–
Yeast species	Campylobacter jejuni				Salmonella sp.			
	30 °C	35 °C	40 °C	45 °C	30 °C	35 °C	40 °C	45 °C
(b)								
<i>Candida pelliculosa</i>	9.0 ± 0.3	–	–	–	5.0 ± 0.2	–	–	–
<i>K. phaffii</i>	–	–	–	–	7.0 ± 0.4	–	–	–
<i>M. pulcherrium</i>	8.0	–	–	–	–	–	–	–
<i>S. cerevisiae</i> 001	25 ± 0.3	–	–	–	–	–	–	–
<i>S. cerevisiae</i> 002	–	–	–	–	19.0	–	–	–
<i>S. cerevisiae</i> 003	–	–	–	–	13.0	–	–	–
<i>R. mucilaginosa</i>	–	–	–	–	5.0 ± 0.1	–	–	–

–, No activity

**Table 6 Effect of salt concentration on the antimicrobial activities of yeast species**

Zone of inhibition (mm)												
Salt conc. (%)	<i>C. palicullosa</i>				<i>K. phaffii</i>				<i>M. pulcherima</i>			
	V.C	L.M	C.J	S	V.C	L.M	C.J	S	V.C	L.M	C.J	S
2%	–	–	–	–	2.0 ± 0.1	–	–	3.0 ± 0.1	2.0 ± 0.1	–	–	2.0 ± 0.3
4%	–	–	–	–	–	–	–	–	–	–	–	–
6%	–	–	–	–	–	–	–	–	–	–	–	–
Control	–	–	9.0 ± 0.2	5.0 ± 0.3	–	–	–	7.0 ± 0.2	11.0 ± 0.2	–	8.0 ± 0.5	–

*Salmonella sp* (S,S), *Campylobacter jejuni* (C.J), *Listeria monocytogenes* (L.M) and *Vibrio cholera* (V.C)

–, No activity

that supported the maximum inhibition zone of 25.0 ± 0.3 mm was recorded by *Saccharomyces cerevisiae* 001 against *Campylobacter jejuni* (Table 5b).

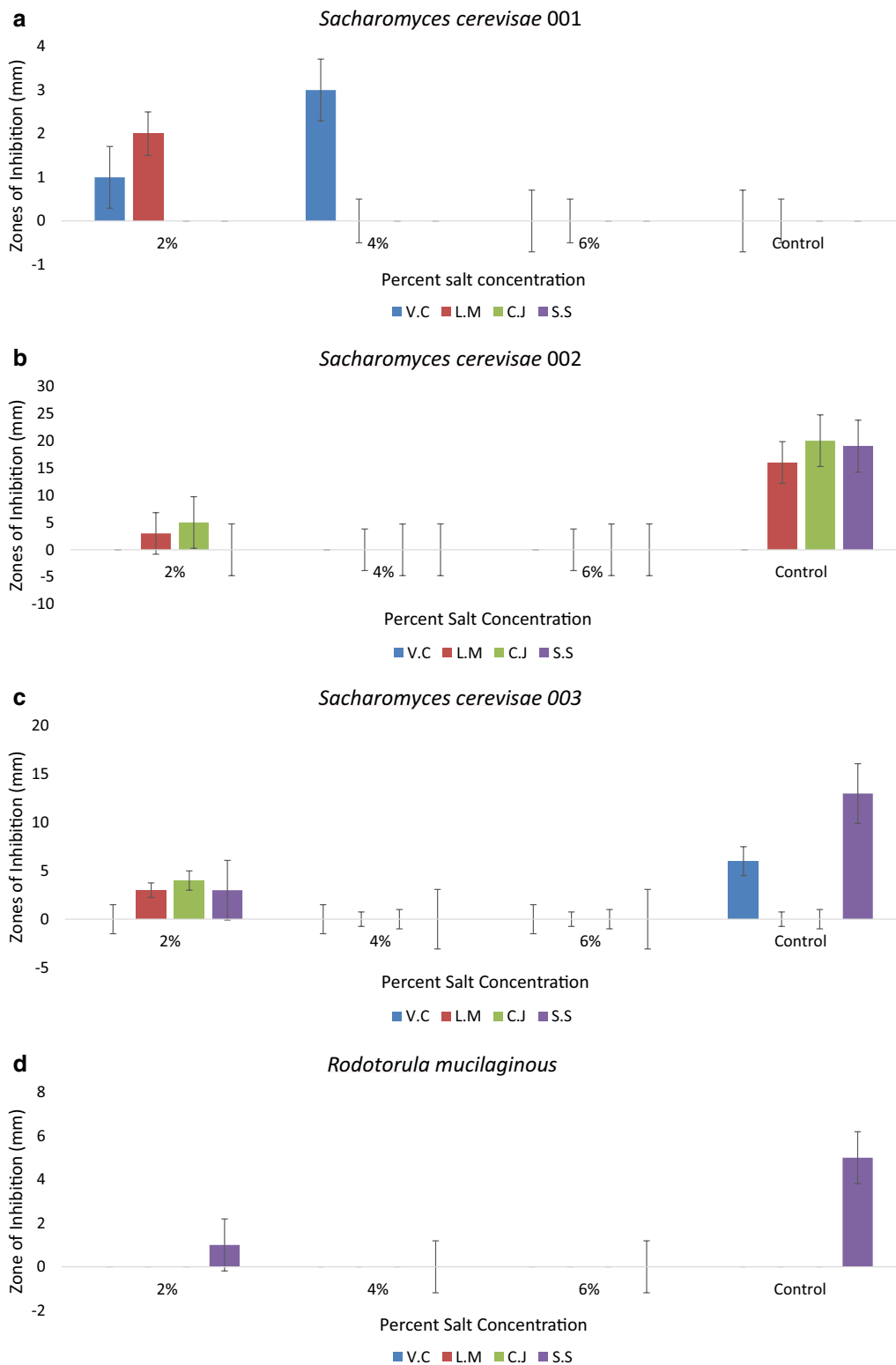
The result of the effect of different sodium chloride concentrations on the antimicrobial activity of yeast species is displayed in Table 6 and Fig. 4a–e. It was observed that 2% sodium chloride concentration stimulated the highest inhibitory performance of 5.0 ± 0.0.1 mm produced by *Saccharomyces cerevisiae* 002 against *Campylobacter jejuni*.

The result of the effect of carbon sources on the antimicrobial activity of yeast species is presented in Table 7 and Fig. 5a–d. The result showed that the supplementation of

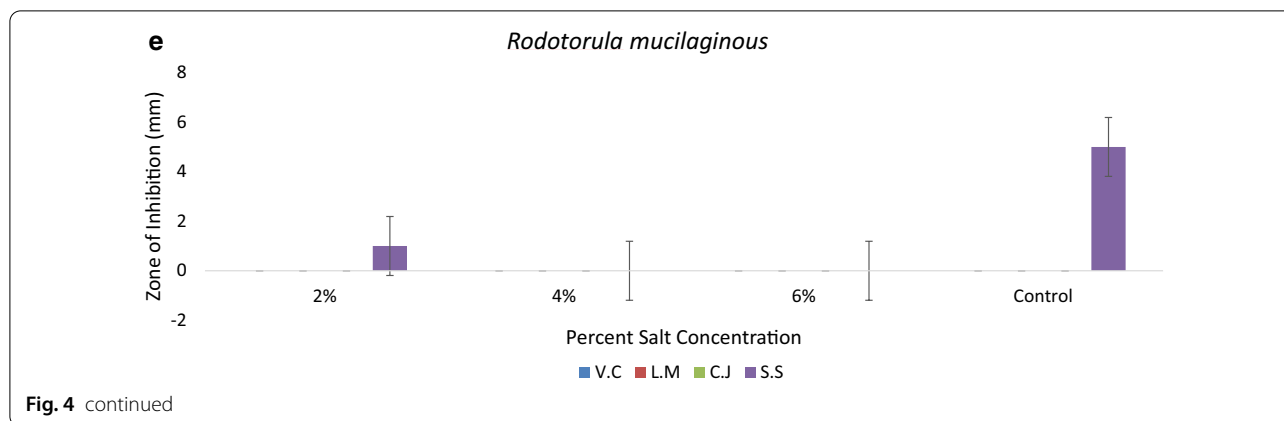
glucose in the growth medium supported the best antimicrobial activity of the yeast species against yeast species with the highest inhibition zone of 19.0 ± 0.1 mm produced by *S. cerevisiae* 001 against *Campylobacter jejuni*, which was lower than the inhibition zone recorded in the control.

The result of the effect of nitrogen sources on the antimicrobial activity of the yeast species against the test bacteria is shown in Table 8a, b and c. It was observed that the supplementation of skimmed milk in the growth medium favoured the best antimicrobial activity of the yeast species with the highest inhibition zone of 27.0 ± 0.2 m recorded by *Saccharomyces* 001 against





**Fig. 4** **a** Effect of salt concentration on the antimicrobial activity of *Saccharomyces cerevisiae*. **b** Effect of salt concentration on the antimicrobial activity of *Saccharomyces cerevisiae* 002. **c** Effect of salt concentration on the antimicrobial activity of *Saccharomyces cerevisiae* 00. **d** Effect of salt concentration on the antimicrobial activity of *Rhodotorula mucilaginosa*. **e** Effect of salt concentration on the antimicrobial activity of *Rhodotorula mucilaginosa*



**Table 7** Effects of carbon source on the antimicrobial activities of yeast isolates

Carbon sources	Zone of inhibition (mm)											
	<i>Candida pelliculosa</i>				<i>Kluyveromyces phaffii</i>				<i>Metschnikowia pulcherrima</i>			
	V.C	L.M	C.J	S.S	V.C	L.M	C.J	S.S	V.C	L.M	C.J	S
Glucose	5.0±0.2	6.0	14.0±0.6	7.0	5.0±0.3	4.0±0.5	2.0±0.1	6.0±0.2	-	-	-	6.0±0.1
Sucrose	-	7.0±0.7	13.0±0.2	6.0±0.3	2.0±0.2	-	-	4.0±0.4	4.0±0.1	6.0	2.0±0.1	7.0±0.2
Fructose	-	5.0	11.0±0.1	5.0±0.1	2.0±0.1	-	3.0±0.1	-	-	-	-	5.0±0.3
Maltose	-	5.0±0.3	12.0±0.6	8.0±0.5	-	-	-	-	-	-	-	5.0
Mannitol	-	4.0±0.7	-	2.0±0.2	-	2.0±0.1	-	-	-	-	2.0	4.0±0.6
Sorbitol	-	18.0±0.3	6.0±0.1	-	-	-	-	-	-	-	-	18±0.6
Xylose	-	11.0±0.2	-	-	-	-	-	-	-	-	-	11±0.1
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-
Control	-	-	9.0±0.6	5.0±0.5	-	-	-	7.0±0.9	11.0±0.2	-	8.0±0.4	-

*Salmonella sp* (S.S), *Campylobacter jejuni* (C.J), *Listeria monocytogenes* (L.M) and *Vibrio cholera* (V.C)

-, No activity

*Campylobacter jejuni* (Table 8b), while an inhibition of 25 mm was observed in the control.

The result of the minimum inhibitory concentration is presented in Table 9. The lowest MIC values of 25.0 ± 0.9 and 25.0 ± 0.8 µg/ml were recorded by *Candida pelliculosa* and *Rhodotorula mucilaginosa*, respectively, against *Salmoella sp*. The highest MIC values of 3.125 ± 0.6 µg/ml and 3.125 ± 0.3 µg/ml were exhibited by *Saccharomyces cerevisiae* 001 against *L. Monocytogenes* and *C. jejuni*. The result of the minimum bactericidal concentration is presented in Table 10. The least MBC values of 6.25 ± 0.2 and 6.25 ± 0.3 were recorded by *Saccharomyces cerevisiae* 001 against *L. Monocytogenes* and *Campylobacter jejuni*, respectively.

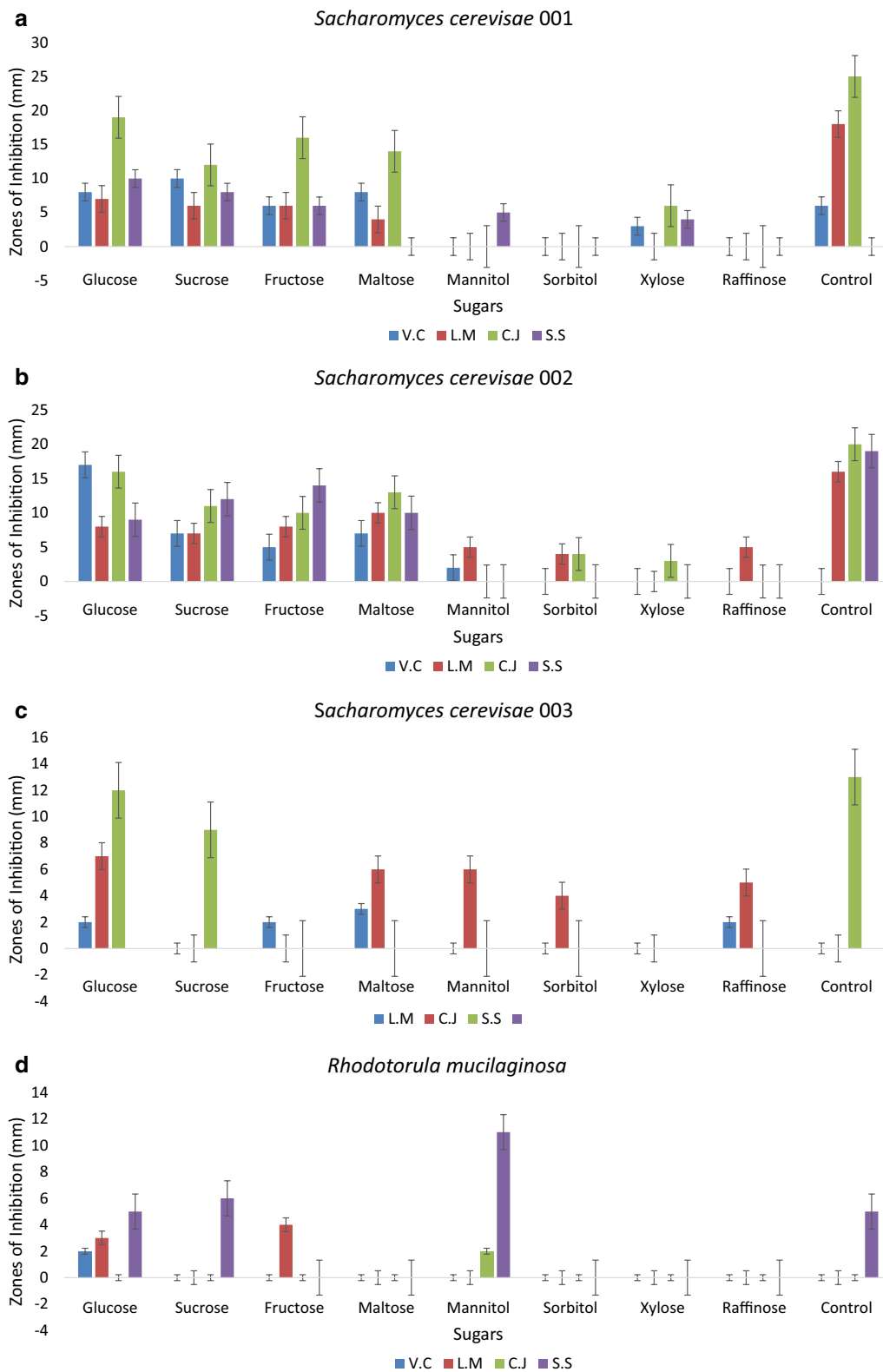
Figure 6 shows the zone on inhibition of *Kluyveromyces phaffii* on *Vibrio cholera*, while Fig. 7 depicts the antagonistic effect of *Metschnikowia pulcherrima* (white arrow),

*Saccharomyces cerevisiae* 001 (red arrow) & 003 (blue arrow) against *Vibrio cholerae*.

Antagonistic effect of *Metschnikowia pulcherrima* on *Salmonella sp*. is shown in Fig. 8, while Fig. 9 represents the effect of *Candida pelliculosa* on *Campylobacter jejuni* at pH 4.

**Discussion**

In this present study, isolation and identification of yeast species and their antimicrobial activity against food-borne pathogens were determined. In addition, effects of some cultural conditions on the inhibitory activity of the yeast species against some selected food-borne pathogens were evaluated. The isolation and identification of yeast species from fruits had been previously documented by Alakeji et al. (2015), Ogunremi et al. (2015), Sulieman et al. (2015), Zerihun (2016) and Fakruddin et al. (2017). Their existence in fruits, fermented foods and beverages is depended on



**Fig. 5** **a** Effect of carbon sources on the antimicrobial activities of *Saccharomyces cerevisiae* 001. **b** Effect of carbon sources on the antimicrobial activities of *Saccharomyces cerevisiae* 002. **c** Effect of carbon source on the antimicrobial activities of *Saccharomyces cerevisiae* 003. **d** Effect of carbon source on the antimicrobial activities of *Rhodotorula mucilaginosa*

**Table 8** Effect of nitrogen sources on the antimicrobial activities of (a) yeast species, (b, c) yeast isolates

Zone of inhibition (mm)												
Nitrogen sources	<i>Candida pelliculosa</i>				<i>Kluyveromyces phaffii</i>				<i>Metschnikowia pulcherrima</i>			
	V.C	L.M	C.J	S.S	V.C	L.M	C.J	S.S	V.C	L.M	C.J	S
(a)												
Alanine	–	5.0±0.3	5.0±0.1	7.0±0.2	2.0±0.2	7.0	2.0±0.1	3.0±0.1	10.0	–	9.0±0.2	4.0±0.4
Aspartic acid	4.0±0.3	–	–	3.0±0.2	–	7.0±0.6	–	2.0±0.1	–	2.0±0.1	–	–
Asparagine	4.0±0.4	2.0±0.1	2.0±0.1	–	–	–	–	–	15.0±0.8	–	5.0±0.6	4.0±0.1
Peptone	–	–	–	6.0±0.4	5.0±0.5	10.00	4.0±0.4	6.0±0.2	10.0	–	–	–
Skimmed milk	2.0±0.1	6.0±0.2	3.0±0.1	5.0±0.3	2.0±0.1	9.0±0.2	2.0±0.1	3.0±0.1	12.0	2.0	2.0±0.1	6.0±0.2
Tryptophan	–	–	–	6.0±0.1	–	–	2.0±0.1	–	9.0±0.5	–	–	–
Control	–	–	9.0±0.5	5.0±0.2	–	–	–	7.0±0.4	11.0±0.7	–	8.0±0.8	–
Nitrogen sources	<i>Saccharomyces cerevisiae</i> 001				<i>Saccharomyces cerevisiae</i> 002							
	V.C	L.M	C.J	S.S	V.C	L.M	C.J	S.S				
(b)												
Alanine	5.0±0.3	16±0.3	23±0.7	5.0±0.1	2.0	17.0±0.6	21.0±0.1	17.0±0.1				
Aspartic acid	7.0±0.1	15.0	24.0±0.1	3.0±0.1	–	19.0	22.0	14.0±0.3				
Asparagine	6.0±0.1	10.0	24.0	–	–	5.0±0.1	11.0±0.3	10.0				
Peptone	5.0±0.2	12±0.1	21.0	–	9.0±0.3	–	19.0±0.2	10.0				
Skimmed milk	4.0±0.1	8±0.2	27±0.2	2.0	7.0±0.4	20.0	17.0±0.1	21.0±0.1				
Tryptophan	5.0	11±0.3	20±0.3	–	5.0±0.3	19.0±0.5	19.0±0.3	15.0±0.6				
Control	–	18±0.1	25±0.6	–	0.2	16.0±0.1	20.0	19.0				
Nitrogen sources	<i>Saccharomyces cerevisiae</i> 003				<i>Rhodotorula mucilaginosa</i>							
	V.C	L.M	C.J	S.S	V.C	L.M	C.J	S.S				
(c)												
Alanine	7.0±0.1	2.0±0.1	3.0±0.1	12.0±0.2	–	5.0±0.1	5.0±0.1	16.0				
Aspartic acid	–	–	–	10.0	–	4.0±0.2	7.0	15				
Asparagine	–	–	–	–	3.0±0.1	–	6.0±0.2	10				
Peptone	–	–	–	–	5.0±0.3	9.0±0.5	5.0±0.1	12.0				
Skimmed milk	12.0±0.5	2.0	4.0±0.2	11.0±0.1	4.0±0.1	7.0±0.3	4.0±0.1	8.0				
Tryptophan	7.0±0.5	–	2.0±0.1	13.0±0.2	–	4.0±0.1	–	–				
Control	6.0±0.4	–	–	13.0	–	–	–	5.0				

*Salmonella sp* (S.S), *Campylobacter jejuni* (C.J), *Listeria monocytogenes* (L.M) and *Vibrio cholera* (V.C)

–, No activity

**Table 9** Minimum inhibitory concentration (µg/ml) of yeast isolates against test organisms

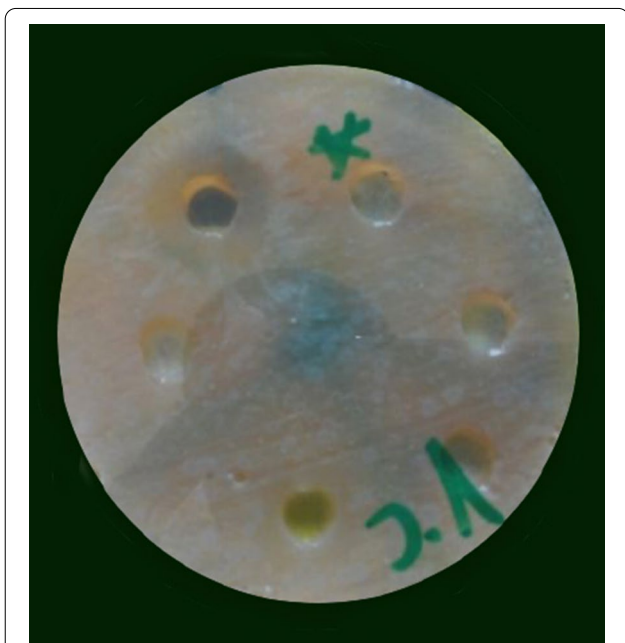
Yeast species	<i>Vibrio cholera</i>	<i>L. Monocytogenes</i>	<i>Campylobacter jejuni</i>	<i>Salmonella sp</i>
<i>Candida pelliculosa</i>	–	–	6.25±0.8	25±0.9
<i>Kluyveromyces phaffii</i>	–	–	–	12.5
<i>Metschnikowia pulcherrima</i>	6.25±0.2	–	12.5±0.7	–
<i>Saccharomyces cerevisiae</i> 001	12.5±0.3	3.125±0.6	3.125±0.3	–
<i>Saccharomyces cerevisiae</i> 002	12.5±0.8	–	–	6.25±0.1
<i>Saccharomyces cerevisiae</i> 003	12.5±0.5	–	–	–
<i>Rhodotorula mucilaginosa</i>	–	–	–	25.0±0.8

–, No activity

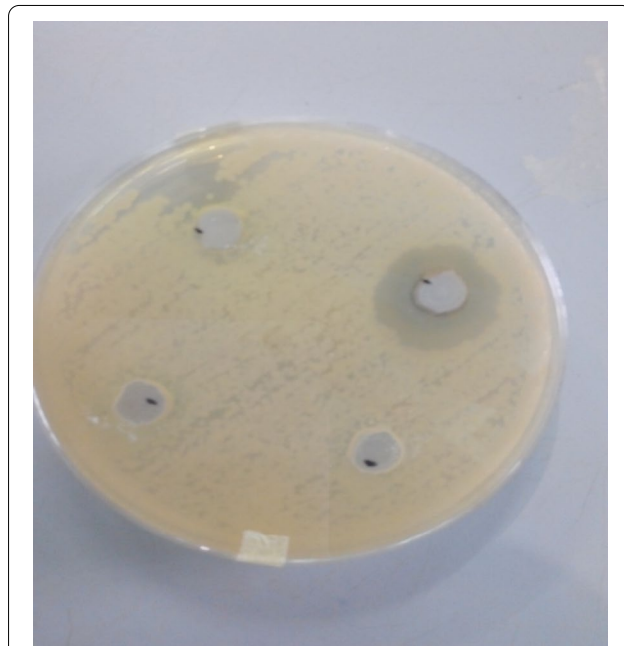
**Table 10** Minimum bactericidal concentration (µg/ml) of yeast supernatant against test bacteria

Isolates code	<i>Vibro cholera</i>	<i>L. Monocytogenes</i>	<i>C. jejuni</i>	<i>Salmonella spp.</i>
<i>Candida palliculosa</i>	–	–	1.25 ± 0.2	50.0
<i>Kluyveromyces phaffii</i>	–	–	–	25.0 ± 0.5
<i>Metschnikowia pulcherrima</i>	12.5 ± 0.2	–	25.5 ± 0.5	–
<i>Saccharomyces cerevisiae</i> 001	25.0 ± 0.2	6.25 ± 0.2	6.25 ± 0.3	–
<i>Saccharomyces cerevisiae</i> 002	25.0 ± 0.5	–	–	12.5 ± 0.6
<i>Saccharomyces cerevisiae</i> 003	25.0 ± 0.3	–	–	–
<i>Rhodotorula mucilaginosa</i>	–	–	–	50.0

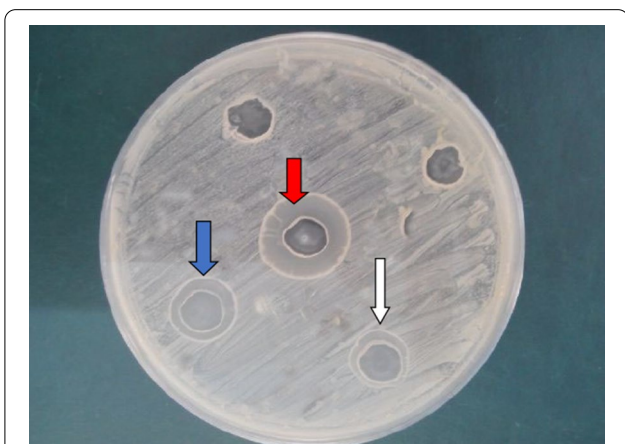
–, No activity



**Fig. 6** Inhibition of *Kluyveromyces phaffii* on *Vibrio cholerae*



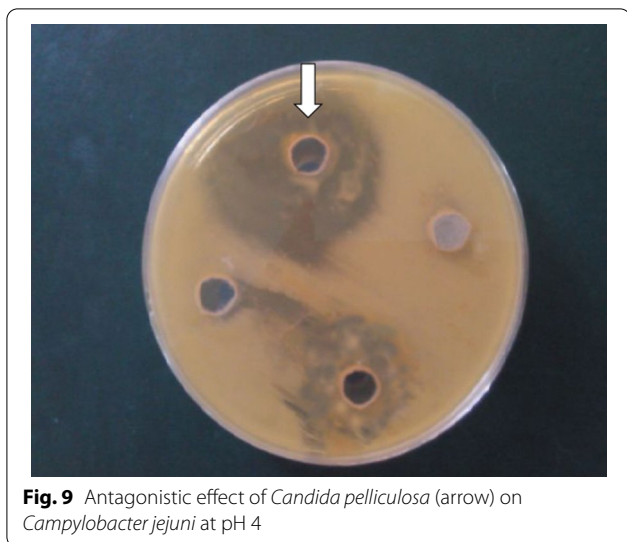
**Fig. 8** Antagonistic effect of *Metschnikowia pulcherrima* on *Salmonella sp*



**Fig. 7** Antagonistic effect of *Metschnikowia pulcherrima* (white arrow), *Saccharomyces cerevisiae* 001 (red arrow) & 003 (blue arrow) against *Vibrio cholerae*

to their high affinity for sugar, their fermentative ability and adaptation to the prevailing conditions in the food matrix and utilization of the nutrients present in the substrate for growth and metabolism.

The identification procedure was carried out by considering their ability to ferment different sugars and nitrogen assimilation test. Sanni (1993), Alakeji et al. (2015) and Fakruddin et al. (2017) had earlier reported the identification of yeast species from traditionally fermented cereal-based products. The diversity of yeast from different fruits as observed in this study is similar to the earlier reports of Sanni and Lonner (1993), Pedersen et al. (2012), Alakeji et al. (2015) and Ogunremi et al. (2015), and reasons such as different geographical location and methods of processing might be adduced



**Fig. 9** Antagonistic effect of *Candida pelliculosa* (arrow) on *Campylobacter jejuni* at pH 4

to be responsible for such occurrences (Sanni and Lonner 1993).

The safety of yeast species has been reported since time immemorial, which confirms their suitability as starter culture in food fermentation. Bourdichon et al. (2012) reported that food-grade yeasts must not be pathogenic as they are generally regarded as safe (GRAS).

The yeast species tested in this present study revealed that all produce lactic acid and diacetyl in varying quantities. The production of inhibitory secondary metabolites by yeast had been previously documented (Marquina et al. 2002; Viljoen 2006). The ability of the yeast species to inhibit the test bacteria is highly dependent on the production of inhibitory metabolites. In addition, it is reported that the inhibitory activity of yeast species had been exploited in bio-control of diseases, probiotic applications, treatment of microbial infections and biotechnological processes (Hatoum et al. 2012). The mechanism of action of the secondary inhibitory compounds produced by yeast species involves changes in the structure and rigidity of the cell wall, thereby causing its dissolution (Dieuleveux et al. 1998).

The inhibitory activity of yeast species against food-borne pathogens observed in this study had been previously reported by Roostita et al. (2011), Younis et al. (2017) and Fakruddin et al. (2017). The ability of the yeast species to inhibit the test bacteria might be due to the production of extracellular protease, secretion of inhibitory proteins, stimulation of immunoglobulin A, acquisition and elimination of secreted toxins, killer toxins or mycocins, sulphur dioxide, hydrogen peroxide, organic acid, volatile acids (Marquina et al. 2002; Viljoen 2006), competition for nutrient, pH changes and production of organic acids (Sigler and Höfer 1991; Buyuksirit and

Kuleasan 2014). The mechanism of action of the secondary inhibitory compounds produced by yeast species involves changes in the structure and rigidity of the cell wall, thereby causing its dissolution (Dieuleveux et al. 1998). It is pertinent to note that the inhibition produced by the yeast species depends on both the antimicrobial compounds secreted by the yeast species and test bacteria (Sousa et al. 2012). It can be inferred from this phenomenon that there is a possible occurrence of a specific strain-dependent antimicrobial effect by the yeast species (Aigner et al. 2006).

In addition, it is observed that the antimicrobial activity of yeast species had been exploited in bio-control of diseases, probiotic applications, treatment of microbial infections and biotechnological processes (Hatoum et al. 2012). According to Czerucka and Rampal (2002), *Saccharomyces boulardii* had been implicated in the prevention of intestinal infections caused by *Escheria coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Yesinia enterocolica* and *Candida albican*. It is also reported that yeast species with resistant traits to antibiotic are very useful in patient receiving antibiotic treatment (Syal and Vohra 2013). The occurrence of low inhibition exhibited by the supernatant of yeast species might infer that the antimicrobial compounds are not extra cellular secreted but cell bound (Georges et al. 2006). In this present study, it was discovered that the yeast species exhibited antimicrobial activity against all the test gram-positive bacteria, but there are reports of inhibition by yeast cell-free supernatant against gram-positive and gram-negative bacteria (Abdelatif et al. 2016). This reported observation is not in agreement with the earlier report of Izgu and Altinbay (1997) who explained that antimicrobial compounds of yeast are only active against gram-positive bacteria.

In addition, Janisiewicz (2010) reported that *M. pulcherrima* is an important yeast antagonist for inhibiting food-borne bacteria on freshly cut fruits based on the possession of pulcherrimin pigment, while Leverentz et al. (2006) confirmed that *M. pulcherrima* did not only prevent the growth, but it even reduced populations of the food-borne pathogens on freshly cut apples. Its inhibitory effects extend to many unrelated species including *C. albicans*.

The highest inhibition zone was recorded at pH of 4, and increasing pH beyond this point showed a reduction in the antimicrobial activities of the yeast metabolites. It could be suggested that high pH value repressed antibacterial activity of the yeast metabolite (Ohenhen et al. 2015). This finding is in conformity with the earlier reports of Richard and Dale (1984), Fadahunsi et al. (2013) and Yoon et al. (2013). However, Soares and Sato (2000) and Al-Qaysi et al. (2017) reported the maximum



activity of *Saccharomyces cerevisiae* at pH range of 4.1–4.5, and this phenomenon might be caused by increased cell surface permeability, resulting in rapid diffusion of nutrients into the cells, which led to increase in the production of antimicrobial substances. In addition, Daeschel and Nes (1991) and Ohenhen et al. (2015) explained that this observation might be caused by the ability of the microorganisms to exhibit a high tolerance at low pH and cell wall membrane physiology. This finding is contrary to the submission of Ohenhen et al. (2015) who reported that diluted crude bacteriocin extract isolated from *Lactobacillus plantarum* exhibited high antimicrobial activity at pH 2 compared to the antimicrobial activity displayed at pH 4, 6 and 8. Saliani et al. (2014) reported higher pH values inhibited bacterial growth by binding to the outside of the bacteria, permeabilizing the outer membrane and disrupting the cytoplasmic membrane. The results obtained in this study showed that the highest zone of inhibition was observed at 30 °C. The observation is in agreement with the submission of Barnett et al. (2000) who reported optimal growth temperature of yeasts between the range of 25–30 °C, and this observation might be responsible for the highest zones of inhibition observed at 30 °C. This apparent expression of antibacterial activity of the yeast metabolites at 30 °C could suggest its stability at this temperature, which negates the findings of Sah et al. (2012) who reported that minimal antibacterial activity of extract was recorded at ambient temperature (28 ± 2 °C). According to Durairaj et al. (2009), the reduction in antimicrobial activity of natural products by heating may be due to volatilization and/or the physical and chemical changes that occur during heating; this submission confirmed the earlier report of Sah et al. (2012) who observed that increasing temperature deteriorated the antibacterial properties of ginger drastically. The effect of increasing temperature showed a decrease in antimicrobial activity and that the zones of inhibition recorded were different for the different yeast species; this finding is similar to the earlier reports of Hajdu (2010) who discovered that increase in temperature to 45 °C, for a prolonged period, may result in thermal burn injuries, while Murray et al. (2003) stated that temperature is a basic requirement for bacterial growth.

The maximum antimicrobial activity of the yeast cell supernatant was enhanced at 2% and 4% NaCl concentration, and beyond this point zero activity was recorded. Similar observation had earlier been reported by Yoon et al. (2013), and the reduced activity recorded might be due to NaCl effect (Hong et al. 2014). Yoon et al. (2013) reported that increase in NaCl concentration decreased antibiotic susceptibility of bacteria to eight antibiotics, and the exposure of bacteria to NaCl may cause variation in antibiotic sensitivity of the bacteria, but the effect

of NaCl on sensitivity of bacteria strains to antibiotic is dependent on both the strain and the antibiotic agent. It was further stated that NaCl might be responsible for increased thermal resistance, cell invasion efficiency and antibiotic resistance of the test bacteria in the food matrix (Yoon et al. 2013). The repression caused by the application of NaCl to antimicrobial sensitivity is linked to Rpos, a general stress response regulator, which is unregulated when bacteria are exposed to osmotic stress, and concluded that NaCl may regulate Rpos expression, thereby affecting antimicrobial sensitivity of bacteria (Hengge-Aronis 2002; Huang et al. 2009). In addition, according to Brejovah (1997), the decrease in the antimicrobial activity due to the effect of sodium chloride at higher concentration in the cultivation medium might have emanated from changes in the composition of extracellular yeast glycoprotein. This report agrees with the earlier findings of Blomberg (2000) who explained that high NaCl concentration imposes both ionic and hyperosmotic stress on yeast cells. However, Al-Qaysi et al. (2017) reported that the maximum antimicrobial activity of killer toxin was recorded at 8% sodium chloride concentration, while the least occurred at 2%, which is inconsistent with the findings of this present investigation.

The highest zone of inhibition was produced when glucose was supplemented into growth medium as the only carbon source, while raffinose stimulated the least. This observation is inconsistent with the submission of Bhattacharyya et al. (1998) who revealed that glycerol supported better antibiotic production by *Streptomyces hygroscopicus*. It was observed that a high degree of variation was observed in the level of antimicrobial activity when the different carbon sources were tested in the medium. It is suggested that the effect of carbon sources on antimicrobial activity is strain dependent. This observation is consistent with the findings of Slinger and Shea-Wilbur (1991) and El-Barnna (2005) who reported that antimicrobial activity of *C. xerosis* NB-2 was highly induced by glucose, and less activity was observed with maltose, sucrose, galactose, ribose, fructose, arabinose and glycerol. According to Gallo and Katz (1972) and Haavik (1974), the presence of glucose in the growth medium is an excellent carbon source for growth because the yeast readily utilizes monosaccharides as the sole carbon sources for their metabolism and interferes with the biosynthesis of many antimicrobial agents such as bacitracin and actinomycin. In addition, glucose is considered the main carbon source by all microorganisms due to its size, rapid uptake, utilization and cellular energy conversion (Buchanan and Stahl 1984; Roitman et al. 1990; Calvo et al. 2002). It is suggested that changes in the fermentation medium development show better positive influence than glucose as carbon sources for

antibiotic production, and nutritional and environmental factors exhibit paramount influence on antibiotic production (El-Barnna 2005). Glucose was also reported as the most suitable carbon source for maximum phenazine production by *Pseudomonas fluorescens* 2-79Q (Slinger and Shea-wilbur 1995). It is pertinent to note that the knowledge of the effect of nutritional composition on antimicrobial substances production could be exploited in process optimization of the production of antimicrobial substances.

The effect of nitrogen sources on the antimicrobial activity of the yeast isolates in this study showed that the highest antimicrobial activity was recorded when skimmed milk was supplemented differently in the growth medium as the sole nitrogen source. There is dearth of available information on the conformity of this finding with previous related existing literature. However, some authors had earlier reported sodium nitrate stimulating the highest activity, Gogoi et al. (2008a, b) reported that ammonium nitrate as nitrogen source promoted the biosynthesis of secondary metabolite, Mahalaxmi et al. (2010) reported similar results for rifamycin B production under SSF using corn husk and wheat bran as the substrate, Ramos and Said (2011) reported that the biosynthesis of secondary metabolites is influenced by cellular condition and that the source of nitrogen is important for the production of antibiotic substances (Demain 1999).

The MIC values of the yeast species metabolites for the test bacteria revealed a pronounced variation. This varying trend is an indication that the MIC values are depended on both the yeast and the test bacteria. The MIC values of the yeast metabolite are measures of potency of the yeast metabolite. Low MIC values indicate high degree of potency of the yeast metabolites, while high MIC values indicate low degree of potency. It is observed that the low MIC and higher MBC values recorded by the metabolites indicated that they are bacteriostatic at lower concentrations and bactericidal at higher concentration (Rahman et al. 2011). It could be suggested that it can be used to inhibit food-borne pathogenic bacteria and be applied in food preservation, bio-control, probiotics application and control of microbial infections. It is pertinent to report that MIC data are very crucial because they can be used as reference point in the treatment of bacterial infections (Aboaba et al. 2011). In addition, it is also observed that the ratios of MBC/MIC were less than or equal to 4, and according to Tepe et al. (2004), the existence of such ratios revealed that the yeast metabolites are bactericidal.

## Conclusion

This study confirmed the antimicrobial activity of yeast metabolites against food-borne pathogens; therefore, based on the production of inhibitory substances they can be considered as a good candidate for use in food preservation.

## Abbreviations

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; GIT: Gastrointestinal tract; YEPD: Yeast extract peptone dextrose.

## Acknowledgements

Not applicable.

## Authors' contributions

IFF designed, conceptualized and supervised the study. SO carried out the experimental protocols and assays. IFF wrote the first draft. The authors read and approved the final manuscript.

## Funding

This research was self-funded.

## Availability of data and materials

All data generated or analysed during this study are included in this article.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

No competing interest exists in the research outcome presented in this article.

Received: 28 October 2020 Accepted: 29 December 2020

Published online: 03 February 2021

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