JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

FUNCTIONAL PROPERTIES OF YEASTS ISOLATED FROM SOME NIGERIAN TRADITIONAL FERMENTED FOODS

Tolulope P. Alakeji[1], *Kolawole Banwo**[1], *Omotade R. Ogunremi*[1] *and Abiodun I. Sanni*[1]

Address(es): Dr. Kolawole Banwo,
[1]University of Ibadan, Faculty of Science, Department of Microbiology, Ibadan, Oyo State Nigeria. +234-8056100840.

*Corresponding author: kolabanwo@yahoo.com

doi: 10.15414/jmbfs.2015.4.5.437-441

ARTICLE INFO

Received 2. 10. 2014
Revised 16. 1. 2015
Accepted 23. 2. 2015
Published 1. 4. 2015

Regular article

OPEN ACCESS

ABSTRACT

Yeasts play important roles in confering some desirable qualities such as nutritional value in traditional fermented foods. This study was carried out to investigate the potentials of yeasts isolated from some Nigerian traditional fermented foods for functional characteristics such as growth at pH 2.5 and 2% bile salts concentration and ability to lower cholesterol in culture medium. A total of 40 yeast strains were isolated from *burukutu*, *ogi* and *pito*. They were characterized phenotypically. Fifteen strains were selected based on the ability to tolerate pH 2.5 and 2% bile salts and they were further identified using API 20C AUX (Biomerieux, France) to be *Debaryomyces hansenii* (5), *Candida krusei* (4), *Candida glabrata* (2), *Candida colliculosa* (1), *Pichia anomala* (1), *Pichia farinosa* (1) and *Pichia membranefaciens* (1). At pH 2.5, *C. glabrata* SA2 showed the highest increase in viable cells count after 24h (6.31 $\log_{10}$ cfu ml^{-1}) while the most sensitive strain was *P. membranefaciens* BA2 (0.70 $\log_{10}$ cfu ml^{-1}). *P. membranefaciens* BA2 survived in 2% bile salts than other yeast strains, with viable cell increase of 0.84 $\log_{10}$ cfu ml^{-1} after 24 h while the least tolerance was observed for *D. hansenii* OA1 with an increase in viable cells of 7.76 $\log_{10}$ cfu ml^{-1}. *C. krusei* OB1 exhibited the greatest reduction of cholesterol of 91.34% while the least reduction of 24.28% was observed for *D. hansenii* OA1 after 48h incubation. The yeast strains in this study demonstrated functional attributes which can be employed as dietary adjuncts for the development of non-dairy beverages with hypocholesterolemic attributes.

Keywords: Yeast, Traditional fermented food, Functional properties, Cholesterol reduction

INTRODUCTION

Fermentation is one of the oldest technologies for food processing and preservation in the world and fermented foods play significant roles in human diet **(Caplice and Fitzgerald, 1999)**. During fermentations, metabolic activities of different groups of microorganisms, including lactic acid bacteria, some yeasts and *Bacillus* species are exploited to improve the safety, nutritional and organoleptic properties of different raw materials such as milk, meats, vegetables, tubers, legumes and cereals **(Odunfa and Oyewole, 1998; Hammes, 2011; Sanni *et al.*, 2013)**. In Africa, traditional fermentation is uncontrolled with the microorganisms either indigenously present on the substrate, from the environment or inoculated from previous successful fermentation batch **(Sanni *et al.*, 1999).** A new trend in food fermentation is to derive health benefits beyond the traditional effects of prolonged shelf-life and improved nutritional value **(Pedersen *et al.*, 2012).** Traditional fermented foods have been identified as flourishing source of microorganisms that have desirable cellular or physiological effects on the body, hence contributing to food functionality **(Champagne *et al.*, 2005).**
Historical evidences supporting the health benefits of functional microbes include, the exceptional longevity observed in the rural populations in Bulgaria and Russian steppes and the reduced serum cholesterol shown by Tribes of Samburu and Massai warriors in Africa after consuming large amount of fermented milk **(Metchnikoff, 1907; Periera and Gibson, 2002).** Several of these health benefits have been demonstrated from *in-vitro* and clinical studies. Although these benefits are strain-specific, functional strains can prevent infections **(Schoster *et al.*, 2013; Rubio *et al.*, 2014),** enhance immune functions **(Geraylou *et al.*, 2013; Patel *et al.*, 2013)** and lower blood cholesterol **(Pan *et al.*, 2011).**
High serum cholesterol level has been implicated in several cardiovascular diseases (CVDs), contributing up to 45% of reported cases **(Baic, 2010).** Cardiovascular diseases will remain the leading causes of death by 2030, affecting approximately 23.6 million people around the world. Therefore, a little reduction in cholesterol will reduce the risk of cardiovascular diseases **(Manson *et al.*, 1992).** Microorganisms with identified health benefits have attracted attention especially as potential cholesterol-lowering agent **(Nguyen *et al.*, 2007; Kobayashi *et al.*, 2012).** A potential functional strain should have the ability to survive conditions of gastrointestinal transit in order to exert the desired health benefits **(Gueimonde and Salminen, 2006).** These strains are usually selected from species of lactic acid bacteria and bifidobacteria but recent efforts are focused towards exploring the larger microbial communities **(Klaenhammer and Kullen, 1999; Galland, 2013).** Yeasts have a long history of safe human consumption as an integral microflora in traditional fermented foods **(Jakobsen and Narhvus, 1996).** They are also prominently used as feed additives to promote growth and enhance performance of livestock **(Auclair, 2001).**
The last decade has recorded few reports exploring the potentials and functionalities of yeast strains with *Saccharomyces boulardii* being the only yeast that has been proven effective and approved for probiotic applications **(Czeruka *et al.*, 2007; Silva *et al.*, 2011, Pedersen *et al.*, 2012; Perricone *et al.*, 2014).** There is a wide physiological diversity of yeasts in African traditional fermented food products **(Sanni and Lonner, 1993)**. The aims of this study were to identify yeast species from some Nigerian traditional fermented foods (*burukutu*, *ogi* and *pito*) and evaluate their functional properties which include acid and bile tolerance, antimicrobial activity and cholesterol reduction ability.

MATERIAL AND METHODS

Yeast enumeration and isolation

Burukutu (4), *ogi* (5) and *pito* (4) samples were aseptically collected from local producers in Ibadan, south-west Nigeria. Appropriate serial dilutions of the samples in sterile peptone water (0.1% w/v) were plated in duplicates onto Malt Extract Agar (MEA) supplemented with chloramphenicol (100 mg l^{-1}). Incubation was carried out at 25 °C for 48 h and yeast count was expressed in $\log_{10}$ cfu ml^{-1} of the sample. Distinct colonies were randomly selected and repeatedly streaked on MEA (without chloramphenicol) until pure cultures were obtained. Isolates were maintained on MEA slants at 4 °C for further use.

Phenotypic characterization

Morphological, biochemical and physiological characterization were carried out using conventional methods.

Morphological characterization

Macro- and micro- morphological characterization was done as described by **Kurtzman *et al.* (2011).** The macroscopic features that were observed include texture, colour, surface, elevation and margin of the colonies. Wet mounts of isolates stained with lactophenol cotton blue were observed using X40 objective of a light microscope. Cellular features observed include cell shape, presence or absence of buds and position of bud(s).

Biochemical and physiological characterization

The physiological tests that were carried include fermentation of various sugars, assimilation of various nitrogen compounds, growth at various temperatures, growth in media with high glucose concentration (50% w/v) and hydrolysis of urea. The carbohydrate fermentation profile of yeast strains was determined and appropriately identified by using API 20C AUX test kit (Biomerieux, France), according to manufacturer's instructions. The fermentation of sugars by yeast isolates was determined as described by **Van der Walt and Yarrow (1984).** The sugars used include glucose, galactose, lactose, maltose, sucrose, xylose and raffinose. Sterile fermentation medium (per liter; 4.5 g of powdered yeast extract and 7.5 g of peptone) was supplemented with filter-sterilized sugar solution to 2% w/v. A positive result was indicated by accumulation of gas in the Durham tubes over a period of seven days. Test for assimilation of different nitrogen compounds was carried out with a basal medium (0.1% potassium dihydrogen phosphate, 0.05% magnesium sulphate heptahydrate, 2% glucose) supplemented with 0.5% of different nitrogen compounds, including, ammonium sulphate, nitrate, lysine and creatinine. Positive result was indicated by an increase in turbidity **(Van der Walt and Yarrow, 1984).** Growth at different temperatures was determined at 25 ^{o}C, 30 ^{o}C, 35 ^{o}C, 37 ^{o}C and 42 ^{o}C. Growth at 50% glucose concentration was determined. Test for hydrolysis of urea was carried out using Christensen's Urea Agar **(Van der Walt and Yarrow, 1984).**

Selection of strains

Yeast strains were screened for the functional properties by growing in Malt Extract Broth with pH adjusted to 3.0, 2.5, 2.0 and 1.5 (3N HCl) and in another broth with different bile salts concentration (0.1, 0.3, 0.5, 1 and 2%). One percent (1% v/v) of 24 h old yeast culture was inoculated into respective Malt Extract Broth and incubated at 37 ^{o}C for 48 h. Increase in turbidity indicated yeast growth **(Psomas *et al.*, 2001).** Strains that showed growth at 2 % bile salts concentration and pH 2.5 were selected for further studies.

Growth at low pH

Fresh culture of selected yeast strains were obtained and inoculated into Malt Extract Broth (1% v/v) with pH adjusted to 2.5 (3N HCl). The broth was incubated at 37 ^{o}C for 72 h and samples were aseptically taken at 0, 24, 48 and 72 h. Viable cell counts were determined from each sample **(Conway *et al*, 1987).**

Tolerance to bile salts

Bile tolerance was determined by inoculating 1% (v/v) fresh culture of selected yeast strains into Malt Extract Broth supplemented with 2% bile salts and incubated at 37^{o}C for 72 h. Viable cells were determined at 0, 24, 48 and 72 h **(Conway *et al*., 1987).**

Antagonistic activity

The antagonistic effect of yeast strains against some foodborne pathogens was determined by the well diffusion method **(Schillinger and Lucke, 1989).** Target organisms (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., *Shigella* sp. and *Pseudomonas aeruginosa*) were supplied from Microbiology Laboratory, University College Hospital, Ibadan, Nigeria. A clear zone of more than 3 mm around the hole was scored positive.

***In vitro* reduction of cholesterol**

The ability of yeast strains to lower cholesterol level *in-vitro* was determined **(Pereira and Gibson, 2002).** Selected yeast strains were inoculated into Malt Extract Broth (1% v/v) supplemented with 0.3% (w/v) of oxgall and 400 µg ml^{-1} of filter-sterilized and water-soluble form of cholesterol (polyoxyethanyl-cholesterol sebacate; Sigma) and incubated at 37 ^{o}C for 48 h. Samples (1 ml) were aseptically taken from the culture at 0, 24 and 48 h. The samples were centrifuged at 18000xg and 4 ^{o}C for 15 min and the supernatant was collected. Cholesterol content of the supernatant was measured with cholesterol test kit (Dialab Diagnostics, Austria). An uninoculated broth served as control and each test was carried out in triplicate. The percentage cholesterol reduction by test yeast strain was calculated using the formula.

Cholesterol reduction (%) = $\frac{Ao-A}{Ao} X\ 100$

Ao: Cholesterol content in uninoculated broth; A: Cholesterol content in culture broth

RESULTS

Enumeration and characterization

The yeasts counts in the samples ranged from 10^4-10^6 cfu g^{-1} after 48-72 h of fermentation (result not shown). A total of 40 yeast strains showing diverse colonial and cellular morphology were isolated from *burukutu* (4), *ogi* (5) and *pito* (4). The colonies of the isolates appeared creamy or whitish on MEA and the cells were round or oval. The yeast isolates fermented different sugar and assimilated some nitrogen compounds. They were presumptively identified to be *Candida* sp. (19), *Saccharomyces* sp. (9), *Debaryomyces* sp. (5), *Pichia* sp. (4), *Torulopsis* sp. (2) and *Brettanomyces* sp. (1). *Pito* samples showed the largest diversity of yeast (Table 1).

Table 1 Distribution of yeast species in some Nigerian traditional fermented foods

Yeast species	*Burukutu* 4[a]	*Ogi* 5[a]	*Pito* 4[a]	Total[b]
***Brettanomyces* sp.**	ND	ND	1	1
***Candida* sp.**	5	6	8	19
***Debaryomyces* sp.**	ND	5	ND	5
***Pichia* sp.**	ND	ND	4	4
***Saccharomyces* sp.**	7	ND	2	9
***Torulopsis* sp.**	ND	1	1	2
Total[c]	12	12	16	40

[a]Values are the number of samples
[b] Number of each yeast species from the Nigerian traditional fermented foods
[c] Number of yeast isolates from each Nigerian traditional fermented food

Selection of strains

The forty yeast strains showed diverse responses to different growth conditions for the selection of functional yeast strains. They all showed different growth levels at pH 3.0 while 21 yeast strains (52.5%) grew at pH 2.5. None of the strain grew at pH 2.0 and 1.5. All the forty strains grew at 0.1% bile salts concentration while 38 (95%), 28 (70%), 28 (70%) and 19 (47.5%) strains showed growth at 0.3, 0.5, 1 and 2% bile salts concentration respectively (result not shown). Fifteen (37.5%) strains were selected based on the ability to grow at pH 2.5 and 2% bile salts concentration and they were identified with API 20C AUX test kit for yeasts identification (Biomerieux, France) to belong to seven species; *Debaryomyces hansenii* (5), *Candida krusei* (4), *Candida glabrata* (2), *Candida colliculosa* (1), *Pichia anomala* (1), *Pichia farinosa* (1) and *Pichia membranefaciens* (1) (Table 2).

Table 2 Identification of selected yeast strains using API 20C AUX test kit

Substrate																			
Glucose	**Glycerol**	**2-Keto-D-gluconate**	**L-Arabinose**	**D-Xylose**	**Adonitol**	**Xylitol**	**Galactose**	**Inositol**	**Sorbitol**	**α-Methyl-D-Glycoside**	**NAcetyl-D-Glucosamine**	**Cellibiose**	**Lactose**	**Maltose**	**Saccharose**	**Trehalose**	**Melezitose**	**Raffinose**	Identification
+	+	+	+	+	-	-	+	-	-	+	-	+	-	+	+	+	+	+	***D. hansenii* (5)[a]**
+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	***C. krusei* (4)[a]**
+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	***C. glabrata*(2)[a]**
+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	***C. colliculosa* (1)[a]**
+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	***P. farinosa* (1)[a]**
+	+	-	-	+	-	-	-	-	-	+	-	-	-	+	+	+	+	-	***P. anomala* (1)[a]**
+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	***P. membranefaciens* (1)[a]**

[a] Values are number of yeast strains selected

Effect of pH 2.5 and 2% bile salts on the growth of yeast strains

Quantitative determination of the effect of different conditions on the test yeast strains revealed species and strain-specific responses. All the selected yeast strains exhibited good growth at pH 2.5 after the intervals of 24 h, 48 h and 72 h. *Candida glabrata* SA2 showed the highest increase in viable cells count of 6.31 $\log_{10}$ cfu ml^{-1} while *P. membranefaciens* BA2 displayed the least increase of 0.70 $\log_{10}$ cfu ml^{-1} at pH 2.5 after 24h of incubation. *Candida colliculosa* PI1 exhibited the best growth with an increase of 6.78 $\log_{10}$ cfu ml^{-1} while *P. membranefaciens* BA2 showed the least of 2.18 $\log_{10}$ cfu ml^{-1} at pH 2.5 after 72 h of incubation. *Pichia membranefaciens* BA2 tolerated 2% bile salts better than other yeast strains, with viable cells increase of 0.84 $\log_{10}$ cfu ml^{-1} while the least tolerance was observed for *D. hansenii* OA1 with an increase in viable cells of 7.76 $\log_{10}$ cfu ml^{-1} after 24h incubation. At 72h incubation, *D. hansenii* OA3 had an increase of 6.25 $\log_{10}$ cfu ml^{-1} while *C. glabrata* SPY3 an increase of 1.88 to 2% bile salt concentration (Table 3).

Antagonistic activity

It was observed that none of the yeast strains showed antagonistic activity against the food borne pathogens using agar well diffusion method (result not shown).

Table 3 Effect of pH 2.5 and 2% bile salts concentration on the growth of selected yeast strains

Yeast strain	Increase in viable cells count after incubation ($\log_{10}$ cfu ml^{-1}) [a]					
	24 h		**48 h**		**72 h**	
	pH 2.5	**2% bile**	**pH 2.5**	**2% bile**	**pH 2.5**	**2% bile**
Debaryomyces hansenii						
OA1	1.93	7.76	3.37	3.92	5.61	2.96
OA2	3.58	4.00	5.02	3.20	4.82	4.24
OA3	2.91	5.85	3.59	7.05	2.67	6.25
OA4	2.54	4.26	3.02	2.46	2.62	1.90
OA5	3.63	6.46	2.83	2.94	2.75	3.78
Candida krusei						
OA6	5.84	5.81	6.52	3.61	5.28	2.85
OB1	5.22	4.03	3.10	4.27	2.98	3.43
OB3	4.97	4.02	4.89	4.68	4.57	4.10
SB2	3.76	4.28	5.52	6.20	5.76	4.36
C. glabrata						
SA2	6.31	4.81	3.81	4.01	6.07	3.53
SPY3	4.23	5.64	4.53	2.92	5.67	1.88
C. colliculosa						
PI1	2.86	6.39	3.66	5.91	6.78	4.15
Pichia farinosa						
SPY1	3.11	2.36	3.71	5.14	5.35	4.36
P. anomala						
SPY2	4.52	5.54	5.08	4.74	6.20	3.14
P. membranefaciens						
BA2	0.70	0.84	1.72	1.80	2.18	4.92

Legend: Increase in viable cells count = $\log_{10}$ cfu ml^{-1} $_{(t\ h)}$ - $\log_{10}$ cfu ml^{-1} $_{(0\ h)}$

***In vitro* reduction of cholesterol**

The selected yeast strains were able to reduce the cholesterol content of the supplemented medium in the presence of 0.3% bile after 24h and 48h. The reduction was within the range of 4.3 to 91.3%. *Debaryomyces hansenii* OA2 displayed the highest reduction of 79.39% while *D. hansenii* OA1 gave the least of 4.3% after 24h. Twelve strains had greater than 50% reduction in cholesterol content after 48h. *Candida krusei* OB1 exhibited the highest reduction of 91.34% while the least reduction of 24.28% was observed for *D. hansenii* OA1after 48h (Fig 1).

DISCUSSION

Many species of yeasts have been reported from different African traditional fermented foods. **Sanni and Lonner (1993)** reported species of *Candida, Saccharomyces* and *Pichia* from *Burukutu*, *Pito* and *Sekete*. *Saccharomyces cerevisiae*, *Candida krusei*, *C. tropicalis*, *Geotrichum candidum*, *G. fermentans* and *Rhodotorula graminis* were isolated during the fermentation of maize for *ogi* production **(Omemu *et al.*, 2007).** They impart characteristic flavours to fermented foods products **(Annan *et al.*, 2003; Jespersen, 2003).** Yeasts also improve the nutritional value of fermented foods **(Hellstrom *et al.*, 2010).** In this study, species of *Brettanomyces*, *Debaryomyces*, *Pichia*, *Saccharomyces* and

Torulopsis were isolated during the fermentation of *burukutu*, *ogi* and *pito*. In this study, *pito* samples had a wide diversity of yeast species which were *Brettanomyces* sp., *Candida colliculosa*, *Candida glabrata*, *Candida krusei*, *Pichia anomala*, *Pichia farinosa*, *Pichia membranaefaciens*, *Saccharomyces cerevisiae* and *Torulopsis* spp. This is not in agreement to a report on the predominance of *Saccharomyces cerevisiae* in *pito* from various regions in Ghana **(Glover *et al.*, 2005).** The difference in the diversity of yeast strains could be due to the type of cereals and ingredient, methods of processing and geographical location **(Sanni, 1993; Sanni and Lonner, 1993).**

The role of yeasts in the development of probiotics has been overlooked even though they occur as integral part of the natural flora of several fermented foods and beverages **(Jakobsen and Narhvus, 1996).** Most food yeasts satisfy the important criteria of safety due to the long history of safe human consumption in traditional fermented food products. For food grade yeasts, there are no reported incidences of opportunistic infections, nor cases of antibiotic resistance due to mobile genetic elements and they do not produce toxic metabolites **(Bourdichon *et al.*, 2012)**. They have the Generally Regarded as Safe (GRAS) status **(FDA, 2001).** The yeasts identified in this study have long history of safety for human consumption in traditional fermented food products. *Debaryomyces hansenii* has been reported in dairy and meat products, where it is important for aroma formation **(Bourdichon *et al.*, 2012)**.

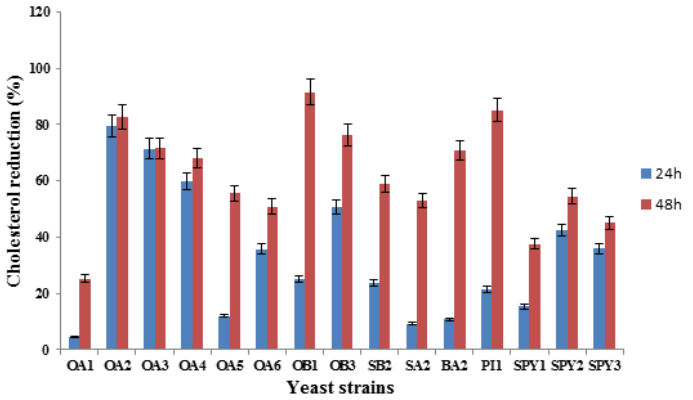

Figure 1 Percentage reduction of cholesterol in broth culture supplemented with 0.3% (w/v) bile salts mixture and 400 µg ml^{-1} cholesterol.

Pichia anomala is a yeast species that is frequently found in food, involved in the fermentation of bakery products and it is proposed for a Qualified Presumption of Safety (QPS) status by European Food Safety Authority **(EFSA, 2007).** Some components of yeast cells are bioactive compounds and several of their metabolic functions are with health beneficial attributes. Bakers yeast glucan and protein isolate from yeasts were reported to prevent the elevation of serum cholesterol **(Robbins and Seeley, 1977).** Important criteria for the selection of probiotic strains are the maintenance of their cell integrity and retaining of their beneficial metabolic functions during gastrointestinal passage **(Klaenhammer and Kullen, 1999)**. There is the need for the strains to survive the natural barriers in the intestine, including body temperature, low pH and elevated bile concentration. The pH in the stomach could be as low as pH 2.5 for 3 hours at fed state due to the release of gastric juice **(Czerucka *et al.*, 2007).** All the yeast strains selected in this study showed satisfactory growth at body temperature of 37°C. The conventional temperature for the growth of most yeast is 25-30°C although some yeasts have been reported to have higher growth temperature **(Kurtzman *et al.*, 2011**). There was variation in the effects of various pH levels and bile concentrations on the growth level of the test isolates. Some yeast strains were able to grow at pH 2.5. Quantitative studies revealed that *Candida colliculosa* PI1 had the best survival rate at pH 2.5. This observation is in agreement with previous reports **(Kumura *et al.*, 2004; Perricone *et al.*, 2014).** Some yeast strains have been reported to tolerate acidic conditions as low as pH 1.5 **(Czerucka *et al.*, 2007).** Strains of *Saccharomyces cerevisiae*, *Candida albicans* and *Debaryomyces hansenii* have survived at pH 3.0 **(Psomas *et al.*, 2001; Kourelis *et al.*, 2010**). In addition, yeasts have been isolated from acidic environments such as lactic acid fermented foods and grape juice **(Omemu *et al.*, 2007; Li *et al.*, 2010; Bonatsou *et al.*, 2015).** All the selected yeast strains increased in colony count at 2 % bile concentration while *Debaryomyces hansenii* OA3 had the best survival. Some yeast strains have been reported to survive in different concentrations of bile during *in-vitro* studies. *Wickerhamomyces anomalus* survived excellently in above 0.6 % bile salts **(García-Hernández *et al*, 2012; Bonatsou *et al.*, 2015)**. Although, bile is a potent antimicrobial compounds, some microorganisms are able to tolerate it due to their bile hydrolase activity or some other bile exclusion mechanisms **(Kumar *et al.*, 2012)**.

One percent reduction in cholesterol can reduce the risk of cardiovascular diseases by 2-3% **(Manson *et al.*, 1992)**. Possible mechanisms for cholesterol removal by probiotic strains are assimilation of cholesterol by growing cells, binding of cholesterol to cellular surface, incorporation of cholesterol into the cellular membrane, deconjugation of bile via bile salt hydrolase, coprecipitation of cholesterol with deconjugated bile, binding action of bile by fibre, and production of short-chain fatty acids by oligosaccharides **(Kumar *et al.*, 2012)**. Yeasts have been noted to remove cholesterol from culture medium, simulating conditions in the intestine by the process of assimilation **(Psomas *et al.*, 2003). Psomas *et al.* (2003)** reported a higher removal of cholesterol by *Isaatchenkia orientalis* KK5.Y.1 and *Saccharomyces* strains. Yeast strains evaluated in this study were able to remove between 4 and 91% of the supplemented cholesterol after 48 hours. *Candida krusei* OB1 showed the highest removal of the supplemented cholesterol.

CONCLUSION

The ability of these yeast strains to tolerate pH 2.5, bile concentration of 2 % and reduction of cholesterol have demonstrated some functional characteristics. They can be used as dietary adjuncts for the development of non-dairy beverages with hypocholesterolemic attributes. However, it is imperative to establish this beneficial effect in *in-vivo* studies.

REFERENCES

ANNAN, N.T., POLL, L., SEFA-DEDEH, S., PLAHAR, W.A. AND JAKOBSEN, M. 2003.Volatile compounds produced by *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough. *Journal of Applied Microbiology*, 94, 462–474.

AUCLAIR, E. 2001. Yeast as an example of the mode of action of probiotics in monogastric and ruminant species. In: *Feed manufacturing in the Mediterranean region. Improving safety: From feed to food*, Brufau J. (ed. Zaragoza: CIHEAM: 45-53.

BAIC, S. 2001. Cholesterol. British Dietetic Association (BDA) Fact Sheet.

BONATSOU, S., BENÍTEZ, A., RODRÍGUEZ-GOMEZ, F., PANAGOU, E.Z. and ARROYO-LOPEZ, F.N. 2015. Selection of yeasts with multifunctional features for application as starters in natural black table olive processing. *Food Microbiology*, 46, 66-73.

BOURDICHON, F., CASAREGOLA, S., FARROKH, C., FRISVAD, J.C., GERDS, M.L., HAMMES, W.P., HARNETT, J., HUYS, G., LAULUND, S., OUWEHAND, A., POWELL, I.B., PRAJAPATI, J.B., SETO, Y., SCHURE, E.T., VAN BOVEN, A., VANKERCKHOVEN, V., ZGODA, A., TUIJTELAARS, S. AND HANSEN, E.B. 2012. Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology*, 154, 87–97. http://dx.doi:10.1016/j.ijfoodmicro.2011.12.030

CAPLICE, E. AND FITZGERALD, G.F. 1999. Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, 50, 131–149.

CHAMPAGNE, C.P., GARDNER, N.J. AND ROY, D. 2005. Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition*, 45(1), 61-84. http://dx.doi.org/10.1080/10408690590900144

CONWAY, P.L. 1987. Selection criteria for probiotic microorganisms. *Asia Pacific Journal of Clinical Nutrition*, 5, 10-14.

CZERUCKA, D., PICHE, T. AND RAMPAL, P. 2007. Review article: yeast as probiotics – *Saccharomyces boulardii*. *Alimentary Pharmacology and Therapeutics*, 26, 767-778. http://dx.doi:10.1111/j.1365-2036.2007.03442.x

EUROPEAN FOOD SAFETY AUTHORITY (EFSA), 2007. Opinion of the scientific committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *The European Food Safety Authority Journal*, 587, 1-16.

FAO, 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria; 2001.

FDA, 2001. Partial list of microorganisms and microbial-derived ingredients that are used in foods.

GALLAND, L. 2013. Functional foods: Health effects and clinical applications. In: *Encyclopedia of Human Nutrition*, Elsevier Ltd. Volume 2: 366-371.

GARCÍA-HERNÁNDEZ, Y., RODRÍGUEZ, Z., BRANDÃO, L.R., ROSA, C.A., NICOLI, J.R., IGLESIAS, A.E., PERÉZ-SANCHEZ, T., SALABARRÍA, R.B. AND HALAIHEL, N. 2012. Identification and *in-vitro* screening of avian yeasts for use as probiotic. *Research in Veterinary Science*, 93(2), 798-802. http://dx.doi:10.1016/j.rvsc.2011.09.005

GERAYLOU, Z., SOUFFREAU, C., RURANGWA, E., DE MEESTER, L., COURTIN, C.M., DELCOUR, J.A., BUYSE, J. AND OLLEVIER, F. 2013. Effects of dietary arabinoxylan-oligosaccharides (AXOS) and endogenous probiotics on the growth performance, non-specific immunity and gut microbiota of juvenile Siberian sturgeon (*Acipenser baerii*). *Fish and Shellfish Immunology*, 35, 766-775. http://dx.doi.org/10.1016/j.fsi.2013.06.014

GLOVER, R.L.K., ABAIDOO, R.C., JAKOBSEN, M. AND JESPERSEN, L. 2005. Biodiversity of *Saccharomyces cerevisiae* isolated from a survey of *pito* production sites in various parts of Ghana. *Systematic and Applied Microbiology*, 28, 755–761. http://dx.doi:10.1016/j.syapm.2005.05.003

GUEIMONDE, M. AND SALMINEN, S. 2006. New methods for selecting and evaluating probiotics. *Digestive and Liver Disease*, 38(2), 242–247.
HAMMES, W.P. 2011. Metabolism of nitrate in fermented meats: The characteristic feature of a specific group of fermented foods. *Food Microbiology*, 1-6. http://dx.doi:10.1016/j.fm.2011.06.016.
HELLSTRÖM, A.M., VÁZQUES-JUÁREZ, R., SVANBERG, U. AND ANDLID, T.A. 2010. Biodiversity and phytase capacity of yeasts isolated from Tanzanian togwa. *International Journal of Food Microbiology*, 136, 352-358. http://dx.doi:10.1016/j.ijfoodmicro.2009.10.011
JAKOBSEN, M. AND NARVHUS, J. 1996. Yeasts and their possible beneficial and negative effects on the quality of dairy products. *International Dairy Journal*, 6:755–768.
JESPERSEN, L. 2003. Occurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African indigenous fermented foods and beverages. *FEMS Yeast Research*, 3, 191-200. http://dx.doi:10.1016/S1567-1356(02)00185-X
KLAENHAMMER, T.R. AND KULLEN, M.J. 1999. Selection and design of probiotics. *International Journal of Food Microbiology*, 50, 45–57.
KOBAYASHI, M., HIRAHATA, R., EGUSA, S. AND FUKUDA, M. 2012. Hypocholesterolemic Effects of lactic acid-fermented soymilk on rats fed a high cholesterol diet. *Nutrients*, 4, 1304-1316. http://dx.doi:10.3390/nu4091304
KOURELIS, A., KOTZAMANIDIS, C., LITOPOULOU-TZANETAKIS, E., SCOURASI, Z.G., TZANETAKIS, N. AND YIANGOU, M. 2010. Preliminary probiotic selection of dairy and human yeast strains. *Journal of Biological Research-Thessaloniki*, 13, 93 – 104.
KUMAR, M., NAGPAL, R., KUMAR, R., HEMALATHA, R., VERMA, V., KUMAR, A., CHAKRABORTY, C., SINGH, B., MAROTTA, F., JAIN, S. AND YADAV, H. 2012. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. *Experimental Diabetes Research*, http://dx.doi:10.1155/2012/902917
KUMURA, H., TANOUE, Y., TSUKAHARA, M., TANAKA, T. AND SHIMAZAKI, K. 2004. Screening of dairy yeast strains for probiotic applications. *Journal of Dairy Science*, 87, 4050-4056.
KURTZMAN, C.P., FELL, J.W., BOEKHOUT, T. AND ROBERT, V. 2011. Methods for isolation, phenotypic characterization and maintenance of yeasts. In: *The Yeasts, a Taxonomic Study*, Elsevier, B.V, 87–110.
LI, S-S., CHENG, C., LI, Z., CHEN, J-Y., YAN, B., HAN, B-Z. AND REEVES, M. 2010. Yeast species associated with wine grapes in China. *International Journal of Food Microbiology*, 138, 85–90.
MANSON, J.E., TOSTESON, H., RIDKER, P.M., SATTERFIELD, S., HEBERT, P., O'CONNOR, G.T., BURING, J.E. AND HENNEKENS, C.H. 1992. The primary prevention of myocardial infarction. *The New England Journal of Medicine*, 326, 1406-1416.
METCHNIKOFF, E. 1907. *Essais optimistes. Paris. The prolongation of life. Optimistic studies*. Translated and edited by P. Chalmers Mitchell. London: Heinemann.
NGUYEN, T.D.T., KANG, J.H. AND LEE, M.S. 2007. Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects. *International Journal of Food Microbiology*, 113, 358–361. http://dx.doi:10.1016/j.ijfoodmicro.2006.08.015
ODUNFA, S.A. AND OYEWOLE, O.B. 1998. African fermented food. In: *Microbiology of Fermented Foods.*Vol.2. Woods BJ. (Eds.). Elsevier Applied Science Publishers London.
OMEMU, A.M., OYEWOLE, O.B. AND BANKOLE, M.O. 2007. Significance of yeasts in the fermentation of maize for ogi production. *Food Microbiology*, 24, 571–576. http://dx.doi:10.1016/j.fm.2007.01.006
PAN, D., ZENGA, X.Q. AND YAN, Y.T. 2011. Characterisation of *Lactobacillus fermentum* SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. *Journal of Science Food Agriculture*, 91, 512–518. http://dx.doi:10.1002/jsfa.4214
PATEL, A.R., SHAH, N.P. AND PRAJAPATI, J.B. 2013. Immunomodulatory effects of probiotics in the treatment of human immunodeficiency virus (HIV) infection. *Biomedicine and Preventive Nutrition*. http://dx.doi.org/10.1016/j.bionut.2013.04.003.
PEDERSEN, L.L., OWUSU-KWARTENG, J., THORSEN, L., AND JESPERSEN, L. 2012. Biodiversity and probiotic potential of yeasts isolated from *Fura*, a West African spontaneously fermented cereal. *International Journal of Food Microbiology*, 159, 144–151. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.08.016
PEREIRA, D.I. AND GIBSON, G.R. 2002. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. *Applied and Environmental Microbiology*, 68, 4689-4693.
PERRICONE, M., BEVILACQUA, A., CORBO, M.R. AND SINIGAGLIA, M. 2014. Technological characterization and probiotic traits of yeasts isolated from Altamura sourdough to select promising microorganisms as functional starter cultures for cereal-based products. *Food Microbiology*, 38, 26-35. http://dx.doi.org/10.1016/j.fm.2013.08.006
PSOMAS, E., ANDRIGHETTO, C., LITOPOULOU-TZANETAKI, E., LOMBARDI, A. AND TZANETAKIS, N. 2001. Some probiotic properties of yeast isolates from infant faeces and Feta cheese. *International Journal of Food Microbiology*, 69, 125–133.
PSOMAS, E.I., FLETOURIS, D.J., LITOPOULOU-TZANETAKI, E. AND TZANETAKIS, N. 2003. Assimilation of cholesterol by yeast strains isolated from infant feces and Feta cheese. *Journal of Dairy Science*, 86, 3416-3422.
ROBBINS, E.A. AND SEELEY, R.D. 1977. Cholesterol loweing effect of dietary yeast and yeast fractions. *Journal of Food Science*, 42, 3, 694-698.
RUBIO, R., JOFRÉ, A., MARTÍN, B., AYMERICH, T. AND GARRIGA, M. 2014. Characterization of lactic acid bacteria isolated from infant faeces as potential probiotic starter cultures for fermented sausages. *Food Microbiology*, 38, 303-311. http://dx.doi.org/10.1016/j.meatsci.2013.09.008
SANNI, A. I., ONILUDE, A.A., OGUNBANWO, S.T. AND SMITH, S.I. 1999. Antagonistic activity of bacteriocin produced by *Lactobacillus* spp. from *ogi*, an indigenous fermented food. *Journal of Basic Microbiology*, 39(3), 189-195.
SANNI, A., FRANZ, C., SCHILLINGER, U., HUCH, M., GUIGAS, C. AND HOLZAPFEL, W. 2013. Characterization and technological properties of Lactic Acid Bacteria in the production of "Sorghurt," a cereal-based product. *Food Biotechnology*, 27(2), 178-198. http://dx.doi.org/10.1080/08905436.2013.781949
SANNI, A.I. 1993. The need for process optimization of African fermented foods and beverages. *International Journal of Food Microbiology* 18, 85 - 95.
SANNI, A.I. AND LONNER, C. 1993. Identification of yeasts isolated from Nigerian traditional alcoholic beverages. *Food Microbio*logy, 10, 517–523.
SCHILLINGER, U. AND LUCKE, F. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. *Applied and Environmental Microbiology*, 55(8), 1901-1906.
SCHOSTER, A., KOKOTOVIC, B., PERMIN, A., PEDERSEN, P.D., DAL BELLO, F. AND GUARDABASSI, L. 2013. *In-vitro* inhibition of *Clostridium difficile* and *Clostridium perfringens* by commercial probiotic strains. *Anaerobe*, 20, 36-41. http://dx.doi.org/10.1016/j.anaerobe.2013.02.006
SILVA, T., RETO, M., SOL, M., PEITO, A., PERES, C.M., PERES, C. AND MALCATA, F.X. 2011. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior. *LWT - Food Science and Technology*, 44, 1349-1354. http://dx.doi:10.1016/j.lwt.2011.01.029
VAN DER WALT, J.P. AND YARROW, D. 1984. Methods for the isolation, maintenance, classification and identification of yeasts. In: *The Yeast: A Taxonomic Study*, Elsevier Amsterdam: 45-105.