Journal of Engineering and Applied Sciences
MEDWELL PUBLICATIONS

Characterisation of Yeasts Isolated from Different Local Fruits and Plant Parts and Their Potential as Probiotics

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Key words: Yeast strains, local fruits and plant parts, PCR-RFLP, probiotic, prebiotic

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Page No.: 2083-2090
Volume: 15, Issue 9, 2020
ISSN: 1816-949x
Journal of Engineering and Applied Sciences

Abstract: Thirteen (n = 13) yeast strains isolated from different local fruits and plant parts were characterised by polymerase chain reaction–restriction fragment length polymorphism analysis. By using four restriction endonucleases (AluI, Hae-III, Hind III and HinfI) on amplicon-Targetedinternal Transcribed Spacer 1 (ITS1) and ITS2 regions, all yeast strains (n =13) were found to be Saccharomycessensuscripto group with Hae III which was the best limiting enzyme to differentiate between *S. cerevisiae/S. paradoxus* (325, 230, 170 and 125 bp) and *S. bayanus/S. postrianus* (495, 230 and 125 bp). Based on 5.8s *rRNA* gene, six yeast strains were identified as *Saccharomyces cerevisiae*, three strains were identified as *S. paradoxus*, and two strains were identified as *S. bayanus* and *S. postrianus* each. Probiotic test was conducted on 13 yeast strains which were grown on bile salt with concentrations from 0.3 to 1%. All strains were also examined for bile deconjugation, lysozyme tolerance and ability to utilise prebiotics. All yeast strains grew in bile salt at 0.3% concentration. However, only *S. cerevisiaevar WU-Y2* (SM16) survived in 1% concentration. In bile deconjugation test, all yeast strains demonstrated reduction in growth and low tolerance towards high concentration of lysozyme (100 ppm). The yeast strains also revealed low growth in inulin, xylitol and lactulose (2% v/w).

INTRODUCTION

'Probiotics' was first conceptualised at the start of the 20th century through Elie Metchnikoff's discovery of the immunological benefits of lactic acid bacteria in fermented milk. Few nonbacterial microorganisms such as yeasts, *Saccharomyces boulardii* and several *Saccharomyces cerevisiae* strains are studied or commercialised as probiotics[1]. Microorganisms must survive passage through the stomach and maintain their viability and metabolic activity in the intestine to be considered as probiotics[2]. Native inhabitants of the

human or animal gastrointestinal tract such as lactobacilli and bifidobacteria, are considered probiotics but often display low stress tolerance which reduce their viability in probiotic applications[2].

Strains of *S. cerevisiae* have been widely tested for probiotic properties, such as protection of bacterial translocation and preservation of gut barrier integrity[3-5]. Different non-Saccharomyces yeast species, especially of the genera *Debaryomyces*, *Torulaspora*, *Kluyveromyces*, *Pichia* and *Candida* have been shown to possess probiotic potential because of their ability to survive and colonise the gastrointestinal tract in different mammalian cell model assays[6]. Probiotic yeasts may also feature inhibitory activities against pathogenic bacteria[7-9]. According to Fleet[10], yeasts have been considered as one of the microorganisms possessing probiotic potential; however, focusing more on livestock production, yeasts are added in feed for ruminants, for example, to increase the weight of animals and milk production. Nevertheless, *S. cerevisiae var. boulardii* is the only yeast with proven clinical effects and probiotic efficiency in double-blind studies[11]. *S. cerevisiae var. boulardii* is used for prevention and treatment of different types of human gastrointestinal diseases[12-16]. McCullough[17] reported that the commercial strains designated as *S.boulardii*should not be considered as separate species from *S.cerevisiae*. Molecular and physiological studies on *S. cerevisiae* and *S. boulardii* by Fietto[18] revealed that *S. boulardii*is genetically very close or nearly identical to *S. cerevisiae*. Other strains of *Saccharomyces* sp. or other yeast genera exhibit similar or better probiotic activity than *Saccharomyces boulardii*[2]. Arroyo-Lopez[19] showed significant interest in finding other yeast strains with probiotic characteristics.

Previously, yeasts isolated from different sources have been characterised by traditional methods[20]. However, these yeasts were not explored with respect to their probiotic diversity. Thus, research on yeasts as probiotics has attracted considerable attention. The present work has aimed to identify the yeast species isolated from different local fruits and plant parts and to characterise new yeast strains with probiotic potential. The yeasts were tested for their abilities to survive in high bile salt and lysozyme concentrations, utilise prebiotics and deconjugate bile salt.

MATERIALS AND METHODS

Yeast isolates: The commercial *S. cerevisiae* strain used in this study was ATCC No. 62418. A total of 30 samples of local fruits and various plant parts were collected from several states of Peninsular Malaysia including Selangor, Pahang, Kelantan and Terengganu. The collected samples were placed aseptically in sterile plastic bags and transferred to ice boxes (4°C) before being transported to the laboratory until use. The selected fruits involved in this study included banana (*Musa acuminate*), papaya (*Carica papaya*), cocoa beans (*Teobroma cacao* L.), palm kernel pulp (*Cocos nucifera* L.), longan (*Dimocarpus longan* spp. *malesianusleenh*), soursop (*Annona muricata* L.), bamboo shoot (*Bambusa vulgaris*), snake fruit (*Salacca zalacca*), jackfruit (Artocarpus heterophyllus), duku Langsat (*Lansium domesticum*), honey, mango (*Mangifer aindica*), durian (*Duriodulcis*), orange (*Citrus sinensis* L.); watermelon (*Citrulus vulgaris*), pineapple (*Ananas comosus*), corn (*Zea mays*); rambutan (*Nephelium lappaceum*); mangosteen (*Garcinia mangostana*), sugar cane juice (*Saccharum officinarum* L.), coconut water (*Cocos nucifera*), fermented rice, niranipah and rice and soil samples. The samples were then subjected to the following procedures within 24-36 h after collection and transferred to the laboratory. Screening tests for the potential yeasts were conducted previously according to the work by Asyikeen *et al*.[20].

Yeast DNA extractions: The yeasts were cultured on Yeast extract-Peptone-Dextrose (YPD) broth for 24 h at 30°C. DNA was extracted using Dneasy® Blood and Tissue Kit (Qiagen, USA) after treatment with lyticase (Sigma, USA). DNA electrophoresis was run in 1.0% (w/v) agarose gels with 1X TAE buffer (40 mMTris-OH, 20 mM acetic acid and 1 mM of ethylenediaminetetraacetic acid; pH 7.6) in a horizontal electrophoresis system (Bio-Rad, USA) for 40 min at a constant current of 100 V. Gels were stained with ethidium bromide and visualised under ultraviolet (UV) light in a transluminator UV (Alpha Innotech, USA) using FluorChem FC2 software (Alpha Innotech, USA).

Yeast characterisation: Yeast DNA was amplified with the primers (internal transcribed spacer 1 (ITS1): TCCGTAGGTGAACCTGCGG and ITS4: TCCTCCGCTTATTGATATGC) as described by Ezteve-Zarzoso *et al*.[21]. Polymerase Chain Reaction (PCR)-DNA sequence amplification was performed in a total reaction volume of 50 μL containing ~50 ng of *DNA* genome in 1 μL of each primer (100 μM), 25 μL of PCR Master Mix (2X) (Fermentas), 10 μL of purified water and 13 μL of nucleus-free water. The thermal cycling parameters comprised an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min and extension at 72°C for 2 min with a final extension at 72°C for 10 min. PCR products were digested with the restriction endonucleases AluI, HaeIII, Hind III and HinfI[22]. Restriction fragments were electrophoresed on 3% agarose gel, stained with

ethidium bromide and photographed. A 100 bp DNA ladder marker (Fermentas, Lithuania) served as the size standard.

DNA sequencing: DNA sequencing was performed by sending 10 μL of amplicons per sample to Olipro Biotechnology Sdn. Bhd. The DNA sequence acquired was then compared with the DNA sequences in the National Center for Biotechnology Information (NCBI) GenBank database through basic alignment search tool (BLAST) software (http://www.ncbi.nlm.nih.gov/BLAST). This analysis determined the DNA sequences featuring high density with sample DNA sequences.

***In-vitro* test for assessing probiotic characteristics:** An in-vitro study was conducted on bile salt, lysozyme tolerance, utilisation of prebiotics and bile salt deconjugation. About 24 h yeast cultures were used for this study. In all the tests, 10^7 cfu mL^{-1} cells were initially maintained in 10 mL of YPD broth before further analysis.

Bile and lysozyme tolerance resistance: The ability of isolated yeast species to grow in the presence of bile salts and lysozyme was studied by Vinderola and Reinheimer[23]. Bile (Sigma) with concentrations of 0.3, 0.5 and 1% (w/v) or 25, 50 and 100 ppm of lysozyme (Sigma) were assayed for each strain. The results were expressed as the percentage of growth (optical density at 600 nm) in the presence of bile or lysozyme compared with the control without addition:

$$\text{Resistance}\% = \frac{TVC_o - TVC_b \times 100}{TVC_o}$$

Utilisation of prebiotics: The ability of the sensitive strains and isolated mutants to ferment prebiotic carbohydrates (lactulose, inulin, raffinose and xylitol; Sigma) was investigated by inoculating the strains (2% v/v) into a modified YPD broth containing prebiotic substrates. The modified YPD broth consisted of basic YPD broth (without glucose) supplemented with 2% (w/v) of each prebiotic. The results were expressed as bile and lysozyme resistance.

Bile salts deconjugation assay: Bile salt deconjugation was determined according to the research by Toranto *et al*.[24]. Sodium salts (Sigma) of Taurocholic (TC) and Tauro DeoxyCholic (TDC) acids were tested against each strain. The presence of an opaque halo of precipitated bile acid around colonies was considered an indication of bile salt deconjugation.

RESULTS AND DISCUSSION

Characterisation of isolated yeasts by PCR-Restriction Fragment Length Polymorphism assay (RFLP): In the present study, PCR-RFLP assay was used for rapid identification of isolated yeasts (Fig. 1). Thirteen yeast colonies isolated from different local fruits and plant parts were analysed. The data obtained previously by physiological and morphological tests allowed the grouping of isolates into Saccharomyces sensustricto[20]. To identify these isolates, a region of the *rRNA* gene repeat unit which includes two noncoding regions designated as ITS1 and ITS2 and the 5.8s *rRNA* gene was amplified and digested by using four restriction endonucleases AluI, Hae III, Hind III and HinfI. The species-specific restriction patterns were obtained and compared with the control (SC) (Table 1).

PCR assay was performed with primer ITS1-and ITS4-amplified PCR products with a size of 850 bp for all yeast isolates. The DNA fragments that resulted from ALU I cleavage of the 850 bp amplicon measured 775 and 75 bp for all isolates, whereas 385, 190 and 175 bp fragments were produced by Hinf I cleavage. Treatment of ITS1 and ITS2 region with AluI, Hind III and HinfI showed no difference in length for all yeast isolates.

Esteve-Zarzoso *et al*.[25] proved that Hae III is the best limiting enzyme to differentiate *Saccharomyces* species. Based on RFLP analysis (Table 1), Hae III cannot distinguish between *S. bayanus/S. postrianus* and *S. cerevisiae/S. paradoxus*. Four fragments were successfully digested from SC, SN1, SKS2, SNR3, SMK9, SDB10, SRB11, SS12, SJ13 and SM16 (325, 230, 170 and 125 bp). Amplification of SB4, SD6, SK14 and SRT15 produced three fragments with different sizes of 495, 230 and 125 bp. In the case of *S. bayanus*, our results showed the presence of two subspecies, namely, S. bayanus internal transcribe spacer 1 and S. bayanusvar 5.8S *rRNa* gene whereas *S. postrianus* included one subspecies (S. pastorianus var weihenstephan). The results of this analysis agree with those of the study by Fernandez *et al*.[26], suggesting that all strains were isolated from local fruits and plant parts belonging to Saccharomyces sensustricto group. The analysis of digestive polymorphisms in the 5.8S rRNA region proved that *S. bayanus* and *S. pastorianus* species are very closely related. This decision is consistent with the study conducted by Vaugh-Martini and Martini[27], Tamai[28] and Yamagishi and Ogata[29].

According to Fernandez-Espinar *et al*.[30], a long controversy exists on the origin of *S. cerevisiae*. Thus, several researchers proposed that this species is a natural organism present in plant fruits[31].

Table 1: Size in bp of PCR products, homology and restriction fragment obtained with four different endonucleases of the major yeast species identified in this study

				Restriction fragment			
Yeast	PCR products ITS1/ITS4	Homology (%)	Yeast identification	Alu I	Hae III	Hind III	HinfI
SN1	850	92	*S. paradoxusvar* ATCC 76856	775+75	325+230+170+125	850	385+190+175
SKS2	850	96	*S. paradoxusvar* 164A	775+75	325+230+170+125	850	385+190+175
SNR3	850	94	*S. cerevisiaevar* ZP 541	775+75	325+230+170+125	850	385+190+175
SB4	850	97	S. bayanus internal transcribe spacer 1	775+75	495+230+125	850	385+190+175
SD6	850	96	*S. bayanusvar* 5.8S *rRNa* gene	775+75	495+230+125	850	385+190+175
SMK9	850	95	*S. cerevisiaevar* EC1118	775+75	325+230+170+125	850	385+190+175
SDB10	850	94	*S. cerevisiaevar* isolate	775+75	325+230+170+125	850	385+190+175
			AUS-LFB-MA-YC2	850	97		
SRB11	850	97	*S. cerevisiaevar* FJ 11	775+75	325+230+170+125	850	385+190+175
SS12	850	92	*S. cerevisiaevar* YN4	775+75	325+230+170+125	850	385+190+175
SJ13	850	98	*S. paradoxusvar isolate* T7B	775+75	325+230+170+125	850	385+190+175
SK14	850	92	*S. pastorianusvarweihenstephan*	775+75	495+230+125	850	385+190+175
SRT15	850	93	*S. pastorianusvar weihenstephan*	775+75	495+230+125	850	385+190+175
SMG16	850	97	*S. cerevisiaevar* WU-Y2	775+75	325+230+170+125	850	385+190+175
SC	850	96	*S. cerevisiaevar* ITS1 (partial)	775+75	325+230+170+125	850	385+190+175

Ladder M: Marker DNA 100 bp; -VE (negative control); SC (positive control) SN1 (pineapple); SKS2 (palm kernel pulp); SNR3 (niranipah); SB4 (papaya); SD6 (dukulangsat); SMK9 (longan) SDB10 (soursop); SRB11 (bamboo shoot); SS12 (snake fruit); SJ13 (corn); SK14 (cocoa beans); SRT15 (rambutan); SM16 (mango)2

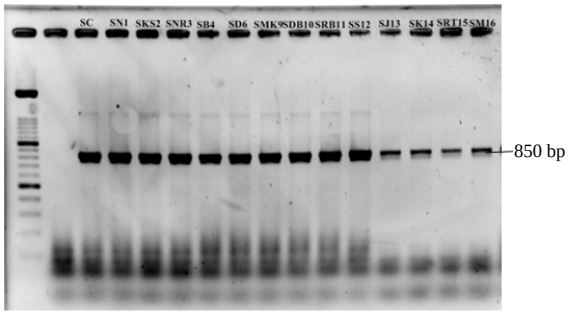

Fig. 1: PCR amplification product line for 14 isolated yeast species from different sources using ITS1/ITS4 primer

Others argued that *S. cerevisiae* is a domesticated species that originated from its closest relative, *S. paradoxus*[27]. The present study confirmed the close relationship between *S. cerevisiae* and *S. paradoxus* which both share the same digested fragments (Table 1). Fernandez-Espinar *et al*. (2003) reported that the *S. cerevisiae* strains isolated from different sources and origins belong to a monophyletic group with a single origin which is compatible with both natural speciation process and single domestication event. Nevertheless, the higher degree of genetic diversity within *S. paradoxus* compared with *S. cerevisiae* is indicative of a more ancient origin of the former species which is compatible with its possible parental role in the origin of *S. cerevisiae*[30].

The yeast strains tested through PCR-RFLP were further analysed by performing DNA sequencing. After obtaining the DNA sequence, these sequences were then compared using the NCBI database through BLAST-nucleotide (BLASTn). BLASTn was used to compare between DNA sequences and identify the region of equality between the target sequence and the highest global order of overall[32]. Table 1 shows the overall data of molecular tests conducted on 14 yeast strains including commercial yeast. The table also shows that the percentage similarity of all isolates exceeded 90%, indicating that all isolates were *Saccharomyces* species.

Six isolates were identified as *S. cerevisiae*, with the different subspecies consisting of *S. cerevisiae*var ZP 541 (pineapple), *S. cerevisiae*var EC1118 (longan), *S. cerevisiae*var isolate AUS-LFB-MA-YC2 (durian), *S. cerevisiae*var FJ 11 (bamboo shoots), *S. cerevisiae*var YN4 (snake fruit) and *S. cerevisiae*var WU-Y2 (mango). The other species successfully isolated from local fruits and plant parts included *S. bayanus*, *S. paradoxus* and *S. postrianus* with different subspecies. All these strains belong to the genus *Saccharomyces*sensustricto[33] which is a very important species in the wine manufacturing industry[34].

Probiotic properties of isolated yeasts: Probiotics are live microorganisms that when administered in adequate amounts, confer health benefits on the host[35]. Pennacchia *et al*.[36] reported that the yeast used as probiotic and belonging to the genus *Saccharomyces* is a subspecies of *Saccharomyces boulardii*. Studies on other yeast species as probiotics has attracted great interest. For the 13 Saccharomyces strains isolated from different local fruits and plant parts, the results corresponded to their loss in cell viability after exposure to bile salt deconjugation, lysozyme and their ability to survive in different types of prebiotic.

Table 2: Effects ofbile salt and deconjugation on the survival of *Saccharomyces* species

	Growth (%) in the presence				
	Bile (%, w/v)			Deconjugation of bile salt	
Yeast strain	0.3	0.5	1.0	TC	TDC
SN1	10.49±0.38	9.69±0.51	8.61±0.51	mg-	mg-
SKS2	9.7088±0.27	9.81±0.41	8.64±0.41	mg-	mg-
SNR3	10.86±0.10	10.48±0.16	10.37±0.11	mg-	mg-
SB4	9.94±0.13	9.82±0.10	9.40±0.30	mg-	mg-
SD6	7.69±0.24	7.44±0.12	7.15±0.21	wg-	wg-
SMK9	7.82±0.15	7.47±0.11	6.23±0.28	wg-	wg-
SDB10	9.96±0.12	6.61±0.37	4.14±0.12	wg-	wg-
SRB11	10.80±0.14	10.54±0.16	9.72±0.20	mg-	mg-
SS12	7.55±0.10	7.39±0.21	7.12±0.10	wg-	ng-
SJ13	7.28±0.49	6.59±0.16	6.59±0.49	vwg-	vwg-
SK14	6.52±0.25	6.24±0.16	6.13±0.13	wg-	wg-
SRT15	12.44±0.22	10.72±0.47	9.39±0.16	mg-	mg-
SM16	9.41±0.33	9.64±0.22	9.72±0.34	mg-	mg-
SC	11.44±0.14	11.25±0.41	10.29±0.95	mg-	mg-

Each value represents the mean value±SD from three trials ng: no growth; vwg: very weak growth; wg: weak growth; mg: moderate growth; g: growth TC, Sodium Taurocholate, TDC: Sodium TaurodeoxyCholate

Bile salt tolerance and its deconjugation: The ability to survive passage through the gastrointestinal tract was investigated by a series of analyses. Table 2 summarises the results (mean±Standard Deviation (SD) of analysis on the effects of bile on the survival of isolated yeasts and their deconjugation. Bile plays a fundamental role in the defence mechanism of the gut and the magnitude of its inhibitory effect is determined primarily by bile salt concentration[37]. In the human gastrointestinal tract, the mean bile concentration is assumed to reach 0.3% w/v which is considered as critical and sufficiently high to screen for resistance[38]. Using this value as a reference in the present study, concentrations of 0.3, 0.5 and 1% were selected to evaluate the growth capability of 14 *Saccharomyces* sp. in the presence of bile salts.

Most of the isolates grew in the medium containing 0.3% bile salt with different levels of resistance but demonstrated a decrease in the percentage of survival except for SM16. As bile salt concentration increased, the growth of *Saccharomyces* species reduced, exhibiting the increasing inhibitory effect of bile salt on growth capability. In the presence of 1% bile salt, the isolates grew from 4.14-10.29% and showed poor bile resistance compared with the results by Pedersen. According to Silva etc. probiotic microorganisms can withstand bile salt concentration of up to 3 g L^{-1}. Bile resistance is an important issue as any ingested microorganism will not reach the intestinal tract in viable form if it cannot resist the presence of bile in the duodenum.

According to Food and Agriculture Organization of the United Nations/World Health Organization (WHO) (2002), deconjugation has been included by the WHO experts as one of the main activities of intestinal microorganisms. Dashkevicz and Feighner[39] reported that the formation of opaque or whitish halo zone around the colony as a result of the release of free bile acids in deconjugation of added bile salts is indicative of the deconjugation ability of an organism. Among the 13 yeast strains, none showed any precipitation/ deconjugation activity on YPD plates supplemented with TC and TDC (Fig. 2). However, all strains grew in the presence of bile salts, coinciding with the data reported by Sourabh *et al*.[40], this result must be ascertained further.

Prebiotic effect and lysozyme resistance: Prebiotic compounds were used with different efficiencies with inulin being the most fermented prebiotic for all the strains assayed except for SDB10. Xylitol and lactulose were poorly fermented by all strains (Table 3). According to Ingrid (2003), a number of criteria must be met for probiotic selection; these criteria include resistance to enzymes in the oral cavity (e.g., lysozyme) and ability to resist digestion in the stomach and intestinal tract.

The lysozyme contained in human saliva[41] and resistance to lysozyme are attributed to the peptidoglycan structure in the cell wall, physiological state of the cell and lysozyme structure in the medium[42]. Among the 13 Saccharomyces strains, 10 were sensitive to lysozyme, with growth percentage of <30%, except for SD6, SMK9, SS12 and SK14 (>30% at 25 ppm) which showed higher ability to grow in the presence of lysozyme. According to Ingrid, the presence of lysozyme in the oral cavity may lyse Gram-positive bacteria. Based on data obtained, all strains showed a decrease in the percentage of survival when lysozyme concentration increased, indicating that this strain may be lysed and may not reach the intestine alive after ingestion. However, the lysozyme concentration used in this study was higher than the physiological intestinal concentration.

Table 3: Characterisation of *Saccharomyces* species on prebiotic and lysozyme tolerance

	Growth (%) in the presence of					
	Prebiotic (2%, w/v)		Lysozyme (ppm)			
Yeast strain	Inulin	Xylitol	Lactulose	25	50	100
SN1	13.33±0.18	11.57±0.38	12.56±0.51	21.08±2.54	16.6±1.27	13.90±1.36
SKS2	14.95±0.27	12.23±0.41	10.87±0.27	23.30±1.37	11.65±1.63	9.71±1.37
SNR3	11.23±0.37	3.06±0.32	4.18±0.21	27.61±3.17	11.19±1.06	1.49±0.32
SB4	12.01±0.40	3.15±0.14	7.49±0.20	14.49±1.00	11.66±1.00	8.13±0.14
SD6	3.12±0.42	2.65±0.12	2.82±0.12	34.19±3.63	34.19±3.63	19.66±2.42
SMK9	3.78±0.11	3.10±0.18	5.30±0.23	40.56±4.54	30.92±2.27	17.27±1.14
SDB10	4.10±0.19	3.65±0.33	10.04±0.25	22.47±2.49	16.29±1.25	12.78±1.25
SRB11	24.15±0.30	7.18±1.00	8.87±0.60	20.88±0.14	19.78±1.55	14.29±0.15
SS12	11.29±0.10	2.56±0.25	3.96± 0.10	41.00±2.04	35.97±1.02	27.34±1.02
SJ13	16.45±0.29	6.56±0.30	3.58±0.16	22.54±4.90	6.36±1.63	1.73±0.49
SK14	8.61±0.71	8.24±0.13	7.44±0.16	35.56±3.14	21.11±1.57	17.78±3.14
SRT15	22.71±0.39	14.20±0.86	14.48±0.47	4.97±0.16	1.65±1.56	0.55±0.11
SM16	14.11±0.17	3.24±0.34	3.95±0.22	16.99±1.12	15.42±1.12	6.72±2.24
SC	13.03±0.34	4.23±0.14	4.94±0.19	19.23±5.44	12.50±1.36	4.81±0.25

Each value represents the mean value±SD from three trials

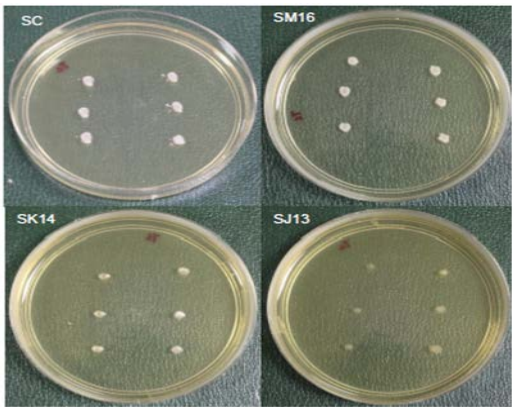

Fig. 2: Survival of *Saccharomyces* species in YPD supplemented with sodium Tauro Cholate (TC)

CONCLUSION

The present findings suggest that all yeasts isolated from different local fruits and plant parts are grouped into the Saccharomycessensustricto. Regarding the presence of bile, all strains showed weak bile resistance at low concentrations. Resistance to bile salts is generally recognised as an essential feature for probiotic strains to survive the conditions in the small intestine[43]. The addition of prebiotics exerted remarkable effects on the survival of yeast species and the count of all isolates decreased significantly. Although, several researchers suggested that yeasts may possess probiotic potential, in the present study, none of the 13 strains evaluated presented this benefit. Overall, our data suggest that yeasts isolated from different local fruits and plant parts are not probiotic. However, from a previous study (data not stated), SMK9, SS12, SRB11 and SMG16 showed good properties as leavening agents compared with commercial yeast and can be candidates for industrial application, especially, in baking technology.

ACKNOWLEDGEMENT

This research was supported by research grants UKM-OUP-NBT-29-138/2011 and Ministry of Education for Mini Budget.

REFERENCES

01. Holzapfel, W.H., P. Haberer, R. Geisen, J. Bjorkroth and U. Schillinger, 2001. Taxonomy and important features of probiotic microorganisms in food and nutrition. Am. J. Clin. Nutr., 73: 365S-373S.
02. Kuhle, A., K. Skovgaard and L. Jespersen, 2005. In vitro screening of probiotic properties of Saccharomyces cerevisae var. boulardii and food borne Saccharomyces cerevisae strains. Int. J. Food Microbiol., 101: 29-39.
03. Martin, F.P.J., Y. Wang, N. Sprenger, E. Holmes, J.C. Lindon, S. Kochhar and J.K. Nicholson, 2007. Effects of probiotic Lactobacillus paracasei treatment on the host gut tissue metabolic profiles probed via magic-angle-spinning NMR spectroscopy. J. Proteome Res., 6: 1471-1481.
04. Klingberg, T.D., U. Lesnik, N. Arneborg, P. Raspor and L. Jespersen, 2008. Comparison of Saccharomyces cerevisiae strains of clinical and nonclinical origin by molecular typing and determination of putative virulence traits. FEMS Yeast Res., 8: 631-640.
05. Generoso, S.V., M. Viana, R. Santos, F.S. Martins and J.A. Machado et al., 2010. Saccharomyces cerevisiae strain UFMG 905 protects against bacterial translocation, preserves gut barrier integrity and stimulates the immune system in a murine intestinal obstruction model. Arch. Microbiol., 192: 477-484.

06. Pedersen, L.L., J. Owusu-Kwarteng, L. Thorsen and L. Jespersen, 2012. Biodiversity and probiotic potential of yeasts isolated from Fura, a West African spontaneously fermented cereal. Int. J. Food Microbiol., 159: 144-151.
07. Kumura, H., Y. Tanoue, M. Tsukahara, T. Tanaka and K. Shimazaki, 2004. Screening of dairy yeast strains for probiotic applications. J. Dairy Sci., 87: 4050-4056.
08. Psani, M. and P. Kotzekidou, 2006. Technological characteristics of yeast strains and their potential as starter adjuncts in Greek-style black olive fermentation. World J. Microbiol. Biotechnol., 22: 1329-1336.
09. Tiago, F.C., F.S. Martins, C.A. Rosa, R.M. Nardi, D.C. Cara and J.R. Nicoli, 2009. Physiological characterization of non-Saccharomyces yeasts from agro-industrial and environmental origins with possible probiotic function. World J. Microbiol. Biotechnol., 25: 657-666.
10. Fleet, G.H., 2007. Yeasts in foods and beverages: Impact on product quality and safety. Curr. Opin. Biotechnol., 18: 170-175.
11. Sazawal, S., G. Hiremath, U. Dhingra, P. Malik, S. Deb and R.E. Black, 2006. Efficacy of probiotics in prevention of acute diarrhoea: A meta-analysis of masked, randomised, placebo-controlled trials. Lancet Infect. Dis., 6: 374-382.
12. Born, P., C. Lersch, B. Zimmerhackl and M. Classen, 1993. The Saccharomyces boulardii therapy of HIV-associated diarrhea. Dtsch. Med. Wochenschr., Vol. 118,
13. Surono, I.S., 2003. In vitro probiotic properties of indigenous Dadih lactic acid bacteria. Asian-Australian J. Anim. Sci., 16: 726-731.
14. Buts, J.P., N. Dekeyser, C. Stilmant, E. Delem, F. Smets and E. Sokal, 2006. Saccharomyces boulardii produces in rat small intestine a novel protein phosphatase that inhibits Escherichia coli endotoxin by dephosphorylation. Pediatr. Res., 60: 24-29.
15. McFarland, L.V., 2007. Meta-analysis of probiotics for the prevention of traveller`s diarrhea. Travel. ed. Infect. Dis., 5: 97-105.
16. Zanello, G., F. Meurens, M. Berri, C. Chevaleyre, S. Melo, E. Auclair and H. Salmon, 2011. Saccharomyces cerevisiae decreases inflammatory responses induced by F4+ enterotoxigenic Escherichia coli in porcine intestinal epithelial cells. Vet. Immunol. Immunopathology, 141: 133-138.
17 McCullough, M.J., K.V. Clemons, J.H. McCusker and D.A. Stevens, 1998. Intergenic transcribed spacer PCR ribotyping for differentiation of Saccharomyces species and interspecific hybrids. J. Clin. Microbiol., 36: 1035-1038.
18. Fietto, J.L., R.S. Araujo, F.N. Valadao, L.G. Fietto and R.L. Brandao *et al.*, 2004. Molecular and physiological comparisons between Saccharomyces cerevisiae and Saccharomyces boulardii. Can. J. Microbiol., 50: 615-621.
19. Arroyo-Lopez, F.N., V. Romero-Gil, J. Bautista-Gallego, F. Rodriguez-Gomez and R. Jimenez-Diaz *et al.*, 2012. Yeasts in table olive processing: Desirable or spoilage microorganisms?. Int. J. Food Microbiol., 160: 42-49.
20. Asyikeen, Z.N., A.G. Ma'aruf, A.M. Sahilah, A.M. Khan and W.W. Aida, 2013. A new source of Saccharomyces cerevisiae as a leavening agent in bread making. Int. Food Res. J., 20: 967-973.
21. Esteve-Zarzoso, B., M.J. Peris-Toran, E. Garcia-Maiquez, F. Uruburu and A. Querol, 2001. Yeast population dynamics during the fermentation and biological aging of sherry wines. Appl. Environ. Microbiol., 67: 2056-2061.
22. Sabate, J., J. Cano, B. Esteve-Zarzoso and J.M. Guillamon, 2002. Isolation and identification of yeasts associated with vineyard and winery by RFLP analysis of ribosomal genes and mitochondrial DNA. Microbiol. Res., 157: 267-274.
23. Vinderola, C.G. and J.A. Reinheimer, 2003. Lactic acid starter and probiotic bacteria: A comparative in vitro study of probiotic characteristics and biological barrier resistance. Food Res. Int., 36: 895-904.
24. Taranto, M.P., F. Seama, A.P.R. Holdago and G.F. Valdez, 1997. Bile salts hydrolase plays a key role on cholesterol removal by Lactobacillus casei. Biotechnol. Lett., 19: 845-847.
25. Esteve-Zarzoso, B., C. Belloch, F. Uruburu and A. Querol, 1999. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers Int. J. Syst. Bacteriol., 49: 329-337.
26. Fernandez, M., J.F. Ubeda and A.I. Briones, 2000. Typing of non-Saccharomyces yeasts with enzymatic activities of interest in wine-making. Int. J. Food Microbiol., 59: 29-36.
27. Martini, A.V. and A. Martini, 1987. Three newly delimited species of Saccharomyces sensu stricto. Antonie Leeuwenhoek, 53: 77-84.
28. Tamai, Y., T. Momma, H. Yoshimoto and Y. Kaneko, 1998. Co-existence of two types of chromosome in the bottom fermenting yeast, Saccharomyces pastorianus. Yeast, 14: 923-933.
29. Yamagishi, H. and T. Ogata, 1999. Chromosomal structures of bottom fermenting yeasts. Syst. Applied Microbiol., 22: 341-353.
30. Fernandez-Espinar, M.T., E. Barrio and A. Querol, 2003. Analysis of the genetic variability in the species of the Saccharomyces sensu stricto complex. Yeast, 20: 1213-1226.

31. Mortimer, R. and M. Polsinelli, 1999. On the origins of wine yeast. Res. Microbiol., 150: 199-204.
32. Claverie, J.M. and C. Notredame, 2003. Bioinformatics for Dummies. 1st Edn., Willey Publishing Inc., New York, ISBN: 0764516965, pp: 480.
33 Martini, A.V. and C.P. Kurtzman, 1985. Deoxyribonucleic acid relatedness among species of the genus Saccharomyces sensu stricto. Int. J. Syst. Evol. Microbiol., 35: 508-511.
34. Skelin, A., S. Sikora, S. Orlic, L. Durakovic and S. Redzepovic, 2008. Genetic diversity of indigenous Saccharomyces sensu stricto yeasts isolated from southern Croatia. Agric. Conspectus Scientificus, 73: 89-94.
35. Guarner, F., G. Perdigon, G. Corthier, S. Salminen, B. Koletzko and L. Morelli, 2005. Should yoghurt cultures be considered probiotic?. Br. J. Nutr., 93: 783-786.
36. Pennacchia, C., G. Blaiotta, O. Pepe and F. Villani, 2008. Isolation of Saccharomyces cerevisiae strains from different food matrices and their preliminary selection for a potential use as probiotics. J. Applied Microbiol., 105: 1919-1928.
37. Charteris, W.P., P.M. Kelly, L. Morelli and J.K. Collins, 2000. Effect of conjugated bile salts on antibiotic susceptibility of bile salt-tolerant Lactobacillus and Bifidobacterium isolates. J. Food Prot., 63: 1369-1376.
38. Gilliland, S.E., T.E. Staley and L.J. Bush, 1984. Importance of bile tolerance of Lactobacillus acidophilus used as a dietary adjunct. J. Dairy Sci., 67: 3045-3051.
39. Dashkevicz, M.P. and S.D. Feighner, 1989. Development of a differential medium for bile salt hydrolase-active Lactobacillus spp. Applied Environ. Microbiol., 55: 11-16.
40. Sourabh, A., S.S. Kanwar and O.P. Sharma, 2011. Screening of indigenous east isolates obtained from traditional fermented foods of Western Himalayas for probiotic attributes. J. Yeast Fungal Res., 2: 117-126.
41. Turchi, B., S. Mancini, F. Fratini, F. Pedonese and R. Nuvoloni *et al.*, 2013. Preliminary evaluation of probiotic potential of Lactobacillus plantarum strains isolated from Italian food products. World J. Microbiol. Biotechnol., 29: 1913-1922.
42. Cunningham, F.E., V.A. Proctor and S.J. Goetsch, 1991. Egg-white lysozyme as a food preservative: An verview. World's Poul. Sci. J., 47: 141-163.
43. Guglielmotti, D.M., M.B. Marco, M. Golowczyc, J.A. Reinheimer and A.D.L. Quiberoni, 2007. Probiotic potential of Lactobacillus delbrueckii strains and their phage resistant mutants. Int. Dairy J., 17: 916-925.