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Short Communication

Comparison of media performances for the recovery of some yeasts from grape juice

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Yeasts are common contaminants of fruit juice concentrates and drinks, carbonated soft drinks, fermented dairy products and high-sugar foods such as syrups. The low pH and high sugar content of these products favor yeast growth resulting in product deterioration (Deak and Beuchat, 1996). Yeast population increases have been reported during and after processing when competitive microorganisms have been either eliminated or their numbers greatly reduced. Thus, raw materials such as contaminated fruit concentrates, serve to initially introduce the yeast (Fugelsang, 1998). In a study undertaken with frozen concentrated orange, apple, cherry, grape and pineapple juices, the most frequently isolated yeast species were reported as *Saccharomyces cerevisiae* (24.7%), *Candida stellata* (22.1%) and *Zygosaccharomyces rouxii* (14.3%) (Deak and Beuchat, 1993). Accurate and rapid detection of yeast contamination in such products is important to guarantee high quality products.

For the isolation of yeasts from foods, there is still a need for a selective medium to ensure the optimum recovery of the yeast species. In this paper, five selective media were evaluated in terms of their suitability to recover and enumerate yeasts. In this study, we also investigated the ability of the media to support colony developments of the yeasts to be found in grape juice.

Three yeast strains; *Zygosaccharomyces rouxii* IFO 0487, *Zygosaccharomyces bailii* IFO 0488 and *Saccharomyces cerevisiae* IFO 2359, used in this study were obtained from the Institute for Fermentation, Osaka (IFO), Japan. Lyophilized yeasts were rehydrated and activated in Yeast extract-Malt extract (YM) broth (LABM^{TM}, UK). The cultures were then grown on YM agar (YM broth plus 1.5% agar) slants at 28°C for 2 days and stored at 4°C until use.

To obtain a natural environment, pasteurized natural white grape juice of a known company in Turkey (Kavaklldere Şarapları A. Ş.), was used as a spiking medium. The grape juice originated from Sultaniye white grapes of the Aegean Region. The composition of grape juice is defined as follows: $4\,g \cdot L^{-1}$ total acidity (equivalent in sulfuric acid), $150\,g \cdot L^{-1}$ sugar, SO_2 of less than $0.5\,mg \cdot L^{-1}$, and no alcohol. pH value of grape juice was determined as 3.5 and brix value was measured as 15.8°Brix. In the experiments, grape juice samples were sterilized by membrane filtration (0.2–0.45 μm, Sartorius, Germany) in order to eliminate the natural flora.

Five selective media recommended for xerophilic fungi were modified and used in this study. In our preworks, it was observed that the incubation periods permitting the visible colony growth were retarded by the increase in sugar content of the media. So, we generally preferred to decrease sugar concentrations in the original compositions. The total sugar concentration

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(w/w) of MY70FG medium (Beuchat and Hocking, 1990) was reduced to 50% and used as MY50FG. The sucrose concentration (w/w) of MY50S, originally recommended by Beuchat (1998), was reduced to 40% and used as MY40S. MYG0.8%P medium was used without making any alteration in its formulation (Tokouka et al., 1985). Another medium (MYG0.1%P) was prepared by decreasing peptone concentration in the original composition of MYG0.8%P. In the preworks of this study, colony formation period on MYG0.8%P was determined to last longer and the size of the colonies was smaller than that of the colonies formed on the other tested media. This was thought to be the effect of the higher peptone concentration in the composition of MYG0.8%P. For this reason, the concentration of peptone was reduced to 0.1% and MYG0.1%P medium was also included in the study. In PDA50SG, originally given as PDA60S (Restaino et al., 1985), sucrose concentration was decreased from 60% to 40% and glucose concentration was increased from 2% to 10% (w/w).

Because MEA has been known as the simplest medium for the enumeration and growth of most food spoilage yeasts, it was used as the control medium (a_w: 0.99, pH: 5.36) in this study. The composition of the other media were as follows: MY50FG (3.0 g malt extract, 3.0 g yeast extract, 5.0 g peptone, 250.0 g fructose, 250.0 g glucose, 6.0 g agar to 500 g distilled water; dw, a_w: 0.89, pH: 5.21); MY40S (20.0 g malt extract, 5.0 g yeast extract, 400.0 g sucrose, 20.0 g agar to 600 g dw, a_w: 0.95, pH: 5.75); MYG0.1%P (3.0 g malt extract, 0.6 g peptone, 3.0 g yeast extract, 400.0 g glucose, 25.0 g agar to 600 ml dw, a_w: 0.93, pH: 5.45); MYG0.8%P (3.0 g malt extract, 5.0 g peptone, 3.0 g yeast extract, 400.0 g glucose, 25.0 g agar to 600 ml dw, a_w: 0.93, pH: 5.40); PDA50SG (250.0 g potato infusion, 100.0 g glucose, 400.0 g sucrose, 9.0 g agar to 250 g dw, a_w: 0.92, pH: 5.21). All of the media were prepared from ingredients (LabM, UK). The media were sterilized by heating in a boiling bath for 30 min with frequent mixing. Thirty percent (w/w) glycerol was used as a dilution medium.

Twenty-four hour-old yeast cultures (4–6$\times 10^6$ cfu$\cdot$ml^{-1}) were separately inoculated into appropriate volumes of grape juice (40–60 ml) to obtain about 1×10^4 cfu$\cdot$ml^{-1}. After inoculation, serial dilutions were made and surface plated onto the media. A parallel study including 24 h pre-incubation at 28°C was also performed after inoculation of the samples. All the plates were incubated at 28°C for 3–7 days. Colony counts and characteristics were recorded. Experiments were performed in duplicate as two replicates of the tested yeast strains. Data were evaluated by variance analysis and Tukey multiple comparison test, using the general linear model of SPSS system.

In this study, while the highest a_w value (0.99) was determined in MEA, MY50FG had the lowest value at 0.89. The pH values of the media were between 5.21–5.75 and in the range permitting the yeast growth.

Comparison of the mean counts were investigated by Tukey multiple comparison test (Table 1). While *Z. rouxii* IFO 0487 and *Z. bailii* IFO 0488 strains were recovered by all of the media, *S. cerevisiae* IFO 2359 strain failed to grow on MY50FG medium during 20 days at 28°C. For *Z. rouxii* strain, the lowest recovery level was obtained with MEA medium at 0-h inoculation. Beside this, the highest recovery was obtained with MY40S medium for the same incubation period. However, no significant differences were found between the recovery levels of any tested media. After a 24 h pre-incubation period in grape juice, MY50FG, MY40S and PDA50SG gave higher recoveries than that of the control medium but no significant differences were observed among the recoveries of these media. The lowest mean count was obtained with MYG0.1%P medium and the difference was found to be significant ($p<0.05$). For *Z. bailii* strain, while MY40S gave the highest recovery in both of the incubation situations, statistically, the differences between MEA and the other media were found to be non-significant at 0 h. When the results were evaluated for 24 h pre-incubation, it could be seen that MY40S was the only medium determining a recovery similar to that of the control medium (Table 1). For *S. cerevisiae* strain, all media had lower recoveries than that of the control medium in both of the incubation situations. For 0 h, mean counts of MEA and MY40S revealed no significant differences. After 24 h pre-incubation in grape juice, the highest recovery level of *S. cerevisiae* strain was obtained with MEA. Statistically, no significant difference was obtained between MYG0.8%P, PDA50SG and MEA.

Data obtained from all yeasts' counts were analyzed by using variance analysis (results are not shown). In the result for 0 h, yeast, media parameters and their interaction were found to be significant. For 24 h pre-incubation period, variance analysis showed that except

Table 1. Comparison of the yeast recovery by different media.

Yeast strain	Media	Recovery performance [log (cfu · ml^{-1})][a]	
		Pre-incubation period	
		0 h	24 h
Z. rouxii IFO 0487	MEA	3.69 a	6.30 a
	MY50FG	3.74 a	6.58 ab
	MY40S	3.83 a	6.52 abc
	MYG0.1%P	3.82 a	6.14 ad
	MYG0.8%P	3.75 a	6.23 acd
	PDA50SG	3.78 a	6.46 abcd
Z. bailii IFO 0488	MEA	4.46 a	7.01 a
	MY50FG	4.31 ab	6.50 b
	MY40S	4.53 ac	7.06 a
	MYG0.1%P	4.48 abc	6.40 b
	MYG0.8%P	4.42 abc	6.40 b
	PDA50SG	4.41 abc	6.59 b
S. cerevisiae IFO 2359	MEA	4.77 a	6.54 a
	MY50FG	—[b]	—[b]
	MY40S	4.76 a	5.59 b
	MYG0.1%P	4.24 b	6.19 c
	MYG0.8%P	4.52 c	6.31 ac
	PDA50SG	4.23 b	6.35 ac

[a] Each value is calculated as the mean of replicates. Values within the same strain and incubation period that are not followed by the same letter are significantly different ($p<0.05$).

[b] No growth detected.

for media replicate interaction all other parameters were statistically significant ($p<0.05$).

On the basis of the results obtained, it appears that for choosing the medium giving the highest recovery performance, more parameters must be considered. For this reason during the study, subjective evaluations concerning the size, color and appearance of colonies, as well as the ease of counting and the incubation period were also recorded (Table 2). One of the disadvantages of a selective medium for yeasts is an incubation period of up to 28 days (Andrews et al., 1997). The most important difference among the modified media was the incubation period permitting the visible colony growth. The shortest incubation time (3 days) was observed with MY40S for all of the tested strains. For the other media, the incubation times varied between 4 and 7 days. When we compared the media according to the size and color of the yeast colonies, MY40S medium was superior to the other four media, by having the largest and most easily observed colonies. The colors of the colonies grown on MYG0.1%P, MYG0.8%P and PDA50SG were very similar to the medium's color and it was difficult to observe them. The color of the colonies grown on MY50FG medium was also close to the medium's color but colonies were also bright. The yeast species *Z. rouxii* and *Z. bailii* both were determined to grow well on MY50FG medium and to produce distinct colonies within 3–8 days at 28°C. However, the difficulty of distinguishing colonies of the size and similar color as well as the long incubation period, has also been recorded as a negative property of the medium. Moreover, *S. cerevisiae* strain tested in our study failed to grow on MY50FG medium. *S. cerevisiae* has been defined as one of the most commonly isolated yeast strains from foods. Because of this, using MY50FG for investigating the yeast flora in foods might result in misleading findings. Additionally, the tolerance of *S. cerevisiae* to low a_w values has been reported to be less than those of the other tested yeast species (Viljoen and Heard, 2000). Hence for *S. cerevisiae*, obtaining higher recoveries on the reference medium (a_w:

Table 2. The subjective comparisons of the media.

Yeast strain	Media	Colony color	Colony diameter (mm)	Colony morphology	Incubation (day)	Ease of counting
Z. rouxii IFO 0487	MEA	Matt/white	4–5	Umbonate	2–3	****
	MY50FG	Glistening/transparent	1–2	Convex	3–6	**
	MY40S	Matt/cream	1–2	Convex	3–5	***
	MYG0.1%P	Matt/cream-white	1–2	Convex	3–5	**
	MYG0.8%P	Matt/cream-white	1–2	Convex	3–5	**
	PDA50SG	Glistening/transparent	2–3	Convex	3–5	***
Z. bailii IFO 0488	MEA	Matt/white	4–5	Umbonate	2–3	****
	MY50FG	Glistening/transparent	<1	Convex	6–8	*
	MY40S	Matt/cream	2–3	Convex	3–5	***
	MYG0.1%P	Glistening/cream-white	1–2	Convex	4–6	**
	MYG0.8%P	Glistening/cream-white	1–2	Convex	4–7	**
	PDA50SG	Matt/grey-green	2–3	Flat	4–5	**
S. cerevisiae IFO 2359	MEA	Matt/cream	4–5	Umbonate	1–3	****
	MY50FG	—[a]	—[a]	—[a]	10–20	—[a]
	MY40S	Matt/white-cream	2–3	Flat	2–3	****
	MYG0.1%P	Glistening/cream-white	<1	Flat	3–5	**
	MYG0.8%P	Glistening/cream-white	<1	Flat	3–5	**
	PDA50SG	Glistening/transparent	1–2	Flat	4–5	***

Ease of counting: ****, very good; ***, good; **, difficult; *, bad.
[a] Colony formation was not detected.

0.99) than those of the other tested media which have lower a_w values, has been thought to be possible.

Another observed disadvantage of the tested media was the difficulty of their preparation. The tested media used in this study all have to be prepared from their ingredients and sterilized in a boiling bath, except the control medium. It has been reported that sterilization of these low a_w and moderately low pH valued media by autoclaving may induce the formation of furfural and other Maillard reaction products (Andrews et al., 1997). However, media used for isolation of yeasts also have been reported to reflect the foods' conditions such as low a_w values and solutes included in the composition of the media (Beuchat, 1998). As a result of this, the difficulty in preparation of the media is thought to be a secondary limiting factor for media development studies.

Among the five selective media tested, MY40S medium was found to give quick and better results for the recovery of the tested yeasts from grape juice. But for supporting the recovery efficiency of the media, this study must also be performed with different yeast strains. Additionally, the performance of the media should be tested with naturally contaminated food samples. In this way, the differentiation capability of the media could be investigated in terms of a mixed microflora.

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