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COMPARISON OF DIFFERENT SELECTIVE MEDIA FOR THE RECOVERY OF SOME YEASTS FROM FRUIT YOGHURT

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Abstract
In this study, different media used for the isolation of some yeasts from spiked fruit yoghurt samples were compared. For this purpose, as control medium; malt extract agar and five selective media were used. These selective media were malt extract yeast extract 50% fructose glucose agar, malt extract yeast extract 40% sucrose agar, malt extract yeast extract glucose 0.8% peptone agar, malt extract yeast extract glucose 0.1% peptone agar and potato dextrose 50% sucrose glucose agar. As yeast strains; *Zygosaccharomyces rouxii* IFO 0487, *Zygosaccharomyces bailii* IFO 0488 and *Saccharomyces cerevisiae* IFO 2359 were used. After suitable incubation periods, recovery performances of the media were determined. Based on statistical evaluation and some subjective parameters, malt extract yeast extract 40% sucrose agar was found superior for the tested strains.

Keywords: Yeast, media, fruit yoghurt, recovery

SEÇİCİ ÖZELLİKTEKİ FARKLI BESİYERLERİNİN BAZI MAYALARIN MEYVELİ YOĞURTTAN GERİ KAZANIMLARI AMACI İLE KARŞILAŞTIRILMALARI

Özet
Bu çalışmada, yapay olarak aşılanan meyveli yoğurt örneklerinden bazı mayaların izolasyonu için kullanılabilecek farklı besiyerleri karşılaştırılmıştır. Bu amaçla, kontrol besiyeri olarak malt extract agar ve beş farklı seçici besiyeri kullanılmıştır. Bu besiyerleri; malt extract yeast extract %50 fructose glucose agar, malt extract yeast extract %40 sucrose agar, malt extract yeast extract glucose %0.8 peptone agar, malt extract yeast extract glucose %0.1 peptone agar and potato dextrose %50 sucrose glucose agar'dır. Maya suşları olarak; *Zygosaccharomyces rouxii* IFO 0487, *Zygosaccharomyces bailii* IFO 0488 ve *Saccharomyces cerevisiae* IFO 2359 kullanılmıştır. Uygun inkübasyon süreleri sonunda, besiyerlerinin geri kazanım özellikleri belirlenmiştir. Yapılan istatistiksel değerlendirmeler ve bazı öznel verilere göre, malt extract yeast extract %40 sucrose agar besiyerinin test edilen suşlar için daha iyi sonuç verdiği tespit edilmiştir.

Anahtar kelimeler: Maya, besiyeri, meyveli yoğurt, geri kazanım

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INTRODUCTION

Yeasts and molds have been reported to become predominant on foods when conditions for bacterial growth get less favorable. Therefore, they could potentially be a problem in fermented dairy products (1). Yeasts are not involved in the fermentation process during yoghurt production, but known as a major cause of spoilage of the final product (2). As they are eliminated from raw milk by prior pasteurization, the presence of yeasts in yoghurt has been reported to be caused by recontamination during manufacturing processes (3). The most common yeast species present in dairy products have been defined as *Kluyveromyces marxianus, Debaryomyces hansenii, Candida famata, Candida kefyr* and other *Candida* species. Also *Rhodotorula mucilaginosa, Yarrowia lipolytica, Torulaspora* and *Pichia* species have been known as prevalent contaminants of dairy industry (4). In recent years the addition of sugar, fruit and flavoring agents to yoghurt has amplified the risk of spoilage by yeasts (5). Because of this, yeasts such as *Zygosaccharomyces* spp. and *S. cerevisiae*, which are known to tolerate low water activity values, can also be responsible of the yoghurt spoilage (5, 6). Additionally, lactose-negative yeasts such as *S. cerevisiae* are often present in yoghurt flora due to the ability to utilize the breakdown products of lactose; glucose and galactose (5).

Yeast enumeration in food was known to be useful to evaluate the quality of food and the degree of deterioration (3). Several methodologies may be used to differentiate the yeast flora of foods. However, it has also been emphasized that new and improved media for selective isolation of various species and strains of yeasts, capable of growing only under specific environmental conditions in specific types of foods, are needed (7, 8).

With this study, we have intended to compare the recovery performances of different selective media for some yeasts by using fruit yoghurt as a spiking medium. The ability of these media to support colony development of the tested yeasts grown in fruit yoghurt was also investigated.

MATERIALS and METHODS

Yeast Strains

Three yeast strains; *Zygosaccharomyces rouxii* IFO 0487, *Zygosaccharomyces bailii* IFO 0488 and *Saccharomyces cerevisiae* IFO 2359 used in this study, were obtained from Institute for Fermentation, Osaka (IFO), Japan.

Fruit Yoghurt

Yoghurt with strawberry pieces, supplied from market, was used as spiking medium. Fruit yoghurt samples were pasteurized at 80 °C for 10 minutes with frequently mixing in a water bath, to eliminate the natural flora. Then the samples were cooled to room temperature before inoculation.

Media and Modifications

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. For this reason, we generally preferred to decrease sugar concentrations in the original compositions. The total sugar concentration (w/w) of malt extract yeast extract 70% fructose glucose (MY70FG) agar medium (9), was reduced to 50% and used as MY50FG. The sucrose concentration (w/w) of malt extract yeast extract 50% sucrose (MY50S) agar, originally recommended by Beuchat (7), was reduced to 40% and used as MY40S medium. Malt extract yeast extract 0.8% peptone (MYG0.8%P) medium was used without making any alteration in its formulation (10). In our preworks, colony formation period on MYG0.8%P was determined to last longer and the size of the colonies was smaller than the colonies formed on the other tested media. This was thought to be the effect of the higher concentration of peptone in the composition of MYG0.8%P. Alternatively, peptone concentration of the medium was reduced and used as malt extract yeast extract 0.1% peptone (MYG0.1%P). In potato dextrose 50% sucrose glucose agar (PDA50SG), originally given as PDA60S (11), sucrose concentration was decreased from 60% to 40% (w/w) and glucose concentration was increased from 2% to 10% (w/w).

Media Preparation

In this study malt extract agar (MEA; 20.0 g malt extract, 1.0 g peptone, 20.0 g glucose, 20.0 g agar

per L) was used as control medium. 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); MY40S (20.0 g malt extract, 5.0 g yeast extract, 400.0 g sucrose, 20.0 g agar to 600 g distilled water); 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 distilled water); 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 distilled water); PDA50SG (250.0 g potato infusion, 100.0 g glucose, 400.0 g sucrose, 9.0 g agar to 250 g distilled water). All of the media were prepared from ingredients (LAB M^{TM}, UK) except potato infusion. Potato infusion was prepared by us according to Pitt and Hocking (12). These media were sterilized by heating in boiling bath for 30 min. 30% (w/w) glycerol was used as dilution medium.

Measurement of Water Activity (a_w) and pH

A_w values of the media were measured with a water activity measurement device, AquaLab Model CX2 (Decagon, USA). The pH values of the media were determined by a pH meter (Jenway 3010, UK).

Inoculation Procedure

During the study, to determine the recovery performances of the media, fruit yoghurt was inoculated with approximately 10^4 cfu/mL for each yeast strain, separately. After inoculation, serial dilutions were made and surface plated onto the media without pre-incubation. This part of the study was accepted as 0 h inoculations. A parallel study including 24 h pre-incubation at 28 °C was also performed after inoculation of the samples. All plates were incubated at 28 °C for 3-20 days. Colony counts and also subjective observations on differences in size, colour, general appearance, and ease of counting colonies on the test media were recorded. Experiments were performed in duplicate as two replicates for each of the tested yeast strain.

Statistical Analysis

Data were evaluated by variance analysis and Tukey multiple comparison test, using SPSS system (13, 14).

RESULTS and DISCUSSION

In this study, while the highest a_w value (0.99) was determined in MEA, MY50FG had the lowest value as 0.89. The pH values of the media were between 5.21-5.75 and in the range permitting the yeast growth (Table 1).

Table 1. A_w and pH values of the media

Media*	a_w	pH
MEA	0.99	5.36
MY50FG	0.89	5.21
MY40S	0.95	5.75
MYG0.1%P	0.93	5.45
MYG0.8%P	0.93	5.40
PDA50SG	0.92	5.21

(*): MEA: malt extract agar; MY50FG: malt extract yeast extract 50% fructose glucose agar; MY40S: malt extract yeast extract 40% sucrose agar; MYG0.1%P: malt extract yeast extract 40% glucose 0.1% peptone agar; MYG0.8%P: malt extract yeast extract 40% glucose 0.8% peptone agar; PDA50SG: potato dextrose 50% sucrose glucose agar

Comparisons of the mean counts were investigated by Tukey multiple comparison test (Table 2). While *Z. rouxii* and *Z. bailii* strains were recovered by all of the media, *S. cerevisiae* strain failed to grow on MY50FG during an incubation period of 20 days at 28 °C. For *Z. rouxii*, recovery levels of the MY50FG and PDA50SG were significantly lower than that of the control medium, at 0 hour. Also recovery levels of MY40S, MYG0.1%P and MYG0.8%P were lower but not significantly different from that of MEA. In the 24 hours-incubated yoghurt samples, there were no significant differences among the recoveries of the media. For *Z. bailii* strain, the best recovery was obtained by MY40S medium, at 0 and 24 hours. At 0 h, the differences between MEA and other media were found to be non-significant. Mean counts revealed no significant differences between MY40S and PDA50SG at 24 h. The major common point of these media is the sucrose, present in their composition. Sucrose has been defined as a better solute than the others for protecting yeasts against negative environmental condition such as low pH (15). For *S. cerevisiae* strain, the differences between mean counts of MEA and

MY40S, and also between MEA and MYG0.8%P were non-significant at 0 hour. However, all of the media except MY40S had lower recoveries than that of MEA. After 24 h incubation in fruit yoghurt, the highest recovery level for *S. cerevisiae* was similarly obtained with MY40S. MYG0.1%P and MYG0.8%P media also had higher recoveries than that of MEA.

Table 2. Comparison of the yeast recovery by different media

Yeasts	Media	Recovery performance [log (cfu/mL)][a] Incubation period (hour) 0	24
Z. rouxii	MEA	4.03a	6.38a
	MY50FG	3.74b	6.38a
	MY40S	3.92ac	6.48a
	MYG0.1%P	3.98ac	6.48a
	MYG0.8%P	3.96ac	6.50a
	PDA50SG	3.90c	6.47a
Z. bailii	MEA	4.25a	6.15a
	MY50FG	4.28a	5.87b
	MY40S	4.32ab	6.27c
	MYG0.1%P	4.20ac	6.14a
	MYG0.8%P	4.24a	6.16a
	PDA50SG	4.29a	6.19ac
S. cerevisiae	MEA	4.74a	6.34a
	MY50FG	-[b]	-[b]
	MY40S	4.85a	6.99b
	MYG0.1%P	4.56b	6.58c
	MYG0.8%P	4.71ab	6.57c
	PDA50SG	4.37b	6.17d

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

[b] : Growth was not detected.

Data obtained from all yeasts' counts were also analyzed by using variance analysis (results are not shown). For 0 hour, all parameters; yeast, media, replicate and their interactions were found significant except replicate and yeast*media*replicate interaction. For 24 h incubation period, variance analysis showed that except replicate all other parameters were statistically significant ($P<0.05$).

In addition to colony count, subjective evaluations were recorded to support statistical results (Table 3). According to the size and colour of the yeast colonies, MY40S medium was found to be superior as having larger and easily observable colonies than the other modified media. In these media (MYG0.1%P, MYG0.8%P and PDA50SG), colours of the colonies were similar to the medium's colour, so it was difficult to observe colony growth. The colour of the colonies grown on MY50FG medium was also close to the medium's colour but colonies were also bright which was making enumeration more difficult. When a study was planned with foods containing a mixed flora, the colour and the brightness of the colonies might be an important factor for differentiating the yeast species. The most important difference among the media was determined as the incubation period permitted to visible colony growth. During the study, the shortest incubation time (3 days) was observed by MY40S for all of the test strains. For the other media, the incubation times were generally varied between 4-7 days.

Table 3. Subjective comparison of the media for the tested yeasts

Yeasts	Media	Colony colour	Diameter (mm)	Morphology	Incubation (day)	Ease of counting
Z. rouxii	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	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	MEA	Matt, cream	4-5	Umbonate	1-3	****
	MY50FG	-	-	-	10-20	-
	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
(-): Growth was not detected.

CONCLUSIONS

Based on the results obtained by statistical analysis and tested subjective evaluations together, MY40S was superior to the other selective media for experimented yeasts in this study. Because of their low pH and sugar content, fruit yoghurts create a selective environment for the growth of yeasts and enable their growth by decreasing a_w and supplying additive carbon source. According to these results, a study with naturally contaminated yoghurt samples must also be performed in order to support the suitability of MY40S for the isolation of the tested yeasts. By this way, the performance of the MY40S medium in means of a mixed microflora, can be investigated.

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