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Original Article

Preparation of Proper Culture Medium for *Saccharomyces cerevisiae var. boulardii* with Molasses and Animal Serum

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Introduction: The purpose of this study was formulation and preparation of a proper culture medium for *Saccharomyces cerevisiae* var. *boulardii* with molasses and animal serum. **Methods:** A fully-crossed factorial design contain 5%, 10% and 20% of molasses (M) with 0, 1% and 5% animal serum (S) was used in this study. The pH of all culture medias were adjusted to 5.6 with acetic acid. The seed was consisted of 10^6 yeast particles which was added to culture media. The inoculated medias were incubated at 37°C for 48 h and the mean yeast counts was recorded. **Results**: The mean of yeast counts for 5% M+0% S, 5% M+1% S, 5% M+5% S, 10% M+0% S, 10% M+1% S, 10% M+5% S, 20% M+0% S, 20% M+1% S, 20% M+5% S were 273333±20033, 228666±34428, 317333±170485, 499333±100425, 516000±38314, 514666±107057, 499333±100425, 516000±38314 and 514666±107057 particles in ml, respectively. In order to optimize culture medium, vitamins and vitamins in combination with minerals (at a concentration of 0.5%) were added to the optimal concentration of molasses and serum. **Conclusion:** Statistical analysis with ANOVA test showed that the growth rate of yeast in 10% molasses plus 1% serum had a significant difference with 5% and 10% molasses in solid medium or 5% molasses supplemented with 1% serum ($p< 0.05$). The addition of vitamins and minerals did not yield significant growth. Therefore, it can be concluded that the combination of molasses and serum may be able to provide basic requirements of the yeast *S. cerevisiae* var. *boulardii*. *J Med Microbiol Infec Dis, 2015, 3 (1-2): 18-22.*

Keywords: Serum, Molasses, *Saccharomyces cerevisiae* var. *boulardii.*

INTRODUCTION

Saccharomyces cerevisiae is one of the best-known members of phylum *Ascomycota*; it is frequently used in the industry and molecular biology [1]. Henri Boulard in 1920 isolated a tropical species of this yeast from the lychee (*Litchi chinensis*) and purple mangosteen (*Garcinia mangostana*) [2]. According to the latest changes in international code name (ICBN), this yeast known as *Saccharomyces cerevisiae* var. *boulardii*. This yeast is one of the most effective probiotics in medicine and veterinary medicine [3].

In Iran sugar beet (*Beta vulgaris*, family: *Chenopodiaceae*) [4] cultivation is around 100,000 hectares and 5,500,000 ton of sugar beet were consumed in 2015 (Iranian Sugar Factory Syndicate, unpublished datas).

Molasses comes from the Latin word; melaseres which means honey-like. It is a dark brown, non-crystallized, inspissated juice from sugar beet, specific gravity of 1.39-1.43 g/cc. Beet molasses comprises sugar, minerals, organic compounds and vitamins [5]. Animal blood is one of the substances that is usually disposed when animals are slaughtered. The animal serum has a total protein equal to 6.74-7.46 g/dl [6].

Most yeasts grow well on commonly used mycological and bacteriological media [7]. Today, several culture mediums are available for yeast propagation, *e.g*. Sabouraud Dextrose Agar (SDA) (a common yeast medium), Minimal Medium (commonly used when testing the mating type of yeast cells), Yeast Extract Peptone Dextrose (a nonselective medium for probiotics), and Rose Bengal Medium (a selective medium which is used for probiotics culture) [8].

However these culture mediums are expensive and hence in practice limit the repeat of the routine laboratory tests. Also, on traditional solid mediums, the sterile collection of yeast is difficult. So, according to the importance of probiotics and the availability of appropriate resources such as molasses and animal byproducts, the purpose of this study was to provide a cheap medium for *S. boulardii* culture. We also discussed the optimization of this medium.

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MATERIAL AND METHODS

Serum and molasses. Beet molasses was prepared from Shahrekord´s sugar factory and was kept at 4°C until used. Blood samples were collected from the animals before slaughtering with 50 ml syringes and transferred to sterile tubes. Then blood samples were centrifuged at 2000 rpm for 5 min and sera were stored at 4°C.

Yeast. lyophilized *S. cerevisiae* var. *boulardii* was purchased from a commercial factory (*leflor*, France) and cultured on SDA for 24 h. The yeast particles were gathered in distilled water and were counted with Neubauer slide.

Design. A fully-crossed factorial design contain 5%, 10% and 20% of molasses (M) with 0, 1% and 5% animal serum (S) was used in this study. The pH of all culture medias adjusted to 5.6 by acetic acid. The seed was consisted of 10^6 yeast particles which was added to culture medias. The inoculated medias were incubated at 37°C for 48 h. and the mean of yeast counts was recorded as the result of cultures. In order to optimize culture media, vitamins (Composition2, table 1) and vitamins in combination with minerals (Composition1, table 1), with 0.5 percent concentration were added to the optimal concentration of molasses and serum combination. The **r**esults of cell counts were analyzed with one-way ANOVA in IBM SPSS statistics 20.

RESULTS

Growth rate in each group with its mean and standard deviation is shown in table 2. The results showed that the lowest growth rate was in 20% Molasses and the highest growth rate relating to the 10% molasses; 1% serum. Statistical analysis with ANOVA test showed that the growth rate of yeast in 10% molasses plus 1% serum has a significant difference with 5% molasses, 10% molasses in solid medium and 5% molasses supplemented with 1% serum ($p< 0.05$).

Table 1. Content of vitamins and vitamins in combination with minerals

Mineral	Composition1	Composition2
Calcium	162 mg	-
Iodine	100 mcg	-
Iron	10 mg	-
Magnesium	50 mg	-
Copper	0. 5 mg	-
Manganese	1 mg	-
Phosphorus	125 mg	-
Potassium	40 mg	-
Chromium	40 mcg	-
Molybdenum	50 mcg	-
Selenium	30 mcg	-
Zinc	5 mg	-
Lutein	250 mcg	-
Bête-carotene (Vit. A)	4000 IU	2500 I.U
Vitamin E	14. 9 IU	15 I.U
Vitamin C	60 mg	60 mg
Folic acid	195 mcg	-
Vitamin B1	1. 4 mg	1 mg
Vitamin B2	1. 6 mg	2.1 mg
Niacin amid (Nicotine amid)	18 mg	5.13 mg
Vitamin B6	2 mg	1 mg
Vitamin B12	1 mcg	-
Vitamin D3	200 IU	400 I.U
Biotin	100 mcg	-
Pantothenic acid	6 mg	-
Vitamin K1	30 mcg	-

Table 2. The counts of yeast cells per ml in each treatment.

Groups	T1	T2	T3	Mean and Standard Deviation
10% Molasses (solid medium)	374000	274500	227000	291833±75017
20% Molasses (solid medium)	138000	294000	514000	315333±188905
5% Molasses + 0% Serum	272000	294000	254000	273333±20033
5% Molasses +1% serum	256000	240000	190000	228666±34428
5% Molasses +5% serum	256000	510000	186000	317333±170485
10% Molasses + 0% serum	624000	530000	426000	526666±99042
10% Molasses + 1% serum	520000	460000	996000	658666±293675
10% Molasses +5% serum	474000	412000	450000	445333±31262
20% Molasses + 0% serum	610000	474000	414000	499333±100425
20% Molasses +1% serum	472000	534000	542000	516000±38314
20% Molasses +5% serum	612000	400000	532000	514666 ± 107057
10% M+1%S+ 0/5% Vit. At 5. 6	344000	328000	238000	303333±57143
10% M+1%S+ 0/5% Vit. At 6. 3	364000	190000	274000	276000±87017
10% M+1%S+ 0/5% vit. + Min. at 5. 6	370000	490000	204000	354666±143615
10% M+1%S+ 0/5% vit. + Min. at 6. 3	246000	342000	426000	338000±90066

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Fig. 1. The growth rate of yeasts in sediment of tubes.
Two left tubes had 5% and 2 right tubes had 20% of molasses.

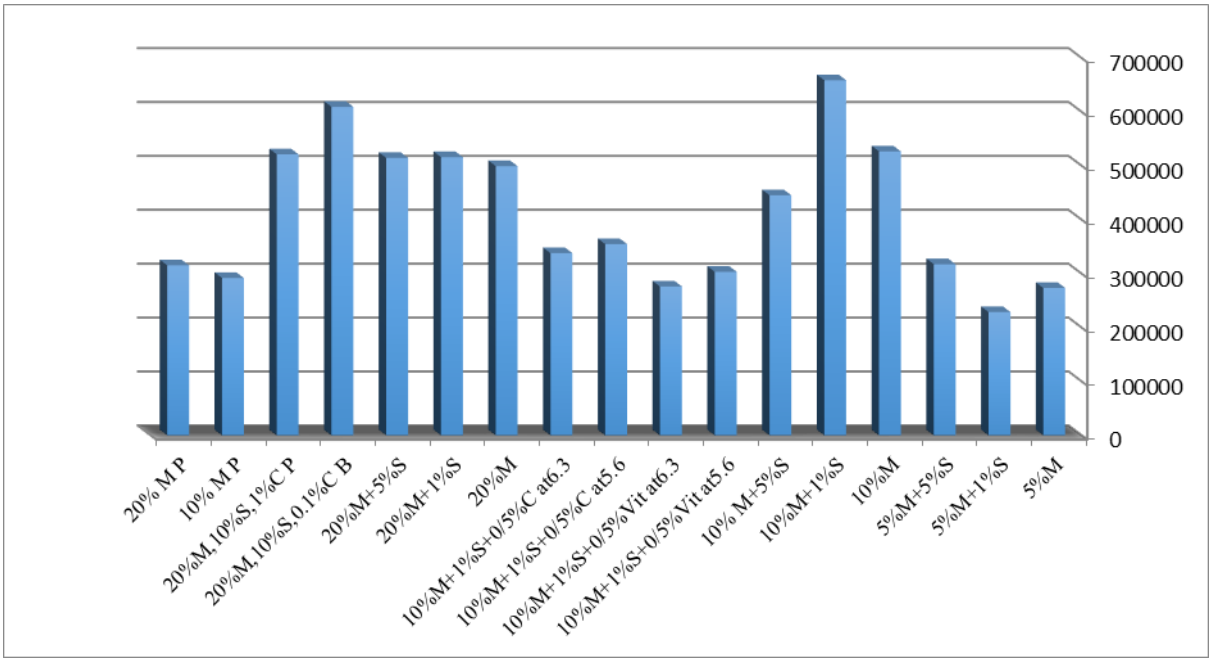


Fig. 2. Comparing the average growth of yeast in each treatment

Table 3. Results of one way ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	838870000000. 000	16	52429375000. 000	3. 655	0. 001
Within Groups	487665833333. 333	34	14343112745. 098		
Total	1326535833333. 333	50			

DISCUSSION

Results showed that number of yeasts in mediums containing 5, 10 and 20 percent were 273,333, 526,666 and 499,333 cells/ml respectively. The ratio between the first and second group was 1.92 and the ratio between the second and third was 1.05, although the difference was not statistically significant, but it seems that the optimal molasses concentration for yeast growth is 10%.

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Addition of serum, in any of the groups (with an equal concentration of molasses) didn't show satisfactory growth. Although in 5% molasses; 1% serum, increasing serum concentration to 5%, showed an increase in yeast growth equal to 1.38 times however there was no statistical difference between 1% and 5% serum treatments. Adding vitamin or vitamin with minerals in any of the groups did not cause significant growth. It may be concluded that the molasses plus serum can supply basic requirements of *S. cerevisiae var.boulardii*.

In order to evaluate the effect of oxygen on yeast growth, growth in the test tubes and plates was compared. In concentrations of 10% molasses, the yeast growth in the test tube was 1.8 more than solid medium. For 20% molasses, this parameter was 1.5 times, but this was not statistically significant. So it can be said that *S. cerevisiae var. boulardii* at concentrations of 10% molasses, in the circumstances of limitation of oxygen have better growth.

For example, Silva and colleagues in 2005, used cane molasses to culture *Escherichia coli K11 and Klebsiella oxytoca P2* [9]. Sarlin and Philip in 2013 used molasses for growing Marine fungi such as: *Fenneropenaeus indicus, Debaryomyces hansenii, Debaryomyces hansenii, Candida sake, Candida tropicalis* [10]. Also Chapman *et al.* (2015) used legume lupin, Nofemele *et al.* (2012) used sugarcane molasses, Batista *et al.* (2013) used extruded bean and Guti *et al.* (2015) used sugarcane bagasse for the growth of *S. cerevisiae* [11, 12, 13, 14].

In this study growth rate of yeast in 10% molasses in test tube was 1.8 times more than the growth rate in the plate. Although this amount was not statistically significant, but it can be said that *S. cerevisiae var. boulardii* has better growth in limitation of oxygen.

Results showed that the mean of growth rate in 10% molasses is 1.92 times of 5% molasses and 1.05 times of 20% molasses. Although this difference was not statistically significant, but it seems that 10% molasses is the optimal concentration of molasses for *S. cerevisiae var. boulardii.*

It has been shown that incubation temperature can affect salt tolerance, perhaps this is true about sugar consumption, too [15]. Though calcium is needed for the massive growth and as a secondary messenger in yeast but Chotineeranat and colleagues in 2010 showed that very high concentration of Ca^{2+} (2.16% w/v) inhibited the growth of the yeast *S. cerevisiae* [1-16]. They express that this is due to inhibition of invertase enzyme. These enzymes are responsible for converting sucrose into glucose and fructose. The potassium and copper ions also have the same effect. In addition, the metal ions have toxic effect on yeast growth by changing the pH and increasing ionic strength [16]. Hasani and the colleagues (2014) showed that the yeast *S. cerevisiae* absorbs copper equal to 16.8 and lead 54.6 mg/gram of dry weight, that is toxic for yeast [17]. Perhaps industrial contamination and the addition of fungicides during the growing and processing of sugar beet, because of limited yeast growth.

Today, increasing laboratory equipment costs made scientists to use inexpensive material such as wastes of agriculture products as a substrate for microbial growth. According to the results it seems that 10% molasses is the optimal concentration of molasses for *S. cerevisiae var. boulardii.* Serum addition is only effective when used with 10% molasses. Thus, we can conclude that protein and sugar concentrations should be set in relation to each other. Adding vitamins or vitamins with minerals did not cause significant growth in any of the groups. Therefore molasses can provide vitamin and mineral requirements of this yeast.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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