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Research Article

PRODUCTION OF SORBITOL BY THE NATIVE YEAST ISOLATED FROM PHYLLOSPHERE

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ABSTRACT
Sorbitol, also known as D-glucitol, is a 6-carbon polyol and its caloric value is similar to glucose; although it is less capable to cause hyperglycemia, since it is converted to fructose in the liver. On the other hand, according to its sweetness and high solubility, the polyol can be applied in food industries as a precursor for many products. The application of microbial process for producing sorbitol has recently become attractive because of mild reaction conditions, being economic and including less environmental problems. The main objective of the present study was to analyze and investigate the production of sorbitol by native yeast isolated from phyllospher. In this study, yeast strain isolated from fig leaf. After identification of the yeast based on its morphological and biochemical properties, the process of fermentation and production of sorbitol from sucrose was investigated. Produced sorbitol was analyzed qualitatively and quantitatively using thin layer chromatography, colorimetric method and kit. *Schizosaccharomyces* sp. produced sorbitol from sucrose after 24hrs. The strain was able to produce 5.71g l^{-1} sorbitol after 96hrs in medium containing 150g l^{-1} sucrose. According to high efficiency of sorbitol production from sucrose by native yeasts, isolation and optimization of production conditions are important.

Keywords: *Microbial Production, Sucrose, Sorbitol, Schizosaccharomyces Sp.*

INTRODUCTION
Sorbitol is a polyol with sweet taste that can be applied as sweetener, softener and a material for absorbing humidity in food industries. Producing vitamin C, sorbose, propylene glycol, synthetic rubber, resin, paper products and textiles are other uses of the mentioned polyol (Barros *et al.*, 2006; Silveira and Jonas, 2002). As the sweetener is not depended on insulin, it can be applied in diet of diabetic people (Barros and Celligoi, 2006). Global production of sorbitol can be estimated more than 500.000ton per year. The substance would be produced commonly through catalytic hydrogenation of di-glucose sap and using nickel catalyst under temperature and pressure (Akinterinwa *et al.*, 2008). After catalytic hydrogenation, the sorbitol would be collected and purified. This step can increase costs of production process (Barros and Celligoi, 2006).
Recently, biotechnological production of sorbitol using microorganisms has been considered as an alternative industrial method (Wei *et al.*, 2001). By 1984, production of sorbitol by *Zymomonas mobilis* bacterium from sucrose or mixture of glucose and fructose was discovered (Viikari, 1984a). Production of sorbitol would be conducted by the bacterium through fructose-glucose oxidoreductase present in the periplasm space of bacterium, which can simultaneously change glucose to glucono-gamma-lactone and fructose to sorbitol (Bekers, 2001; Springer, 1996). Produced sorbitol would be concentrated in the periplasm, so that it can protect cells from harmful effects of high osmosis pressure of the ambient (Barros and Celligoi, 2006). Yeasts have been also applied since long times in different industries and food industry and they have been known as healthy resources because of their non-pathogenic properties (Vakhlu and Kour, 2006). Biological, chemical and physical factors interfere in distribution of yeasts and can make them occupy various habitats (Kurtzman and Fell, 1998). Because of photosynthetic ability of plants and production of many carbonate compounds, plants can provide suitable habitats for different species of yeasts. Different parts of plants like their leaf blade can be considered as special places for yeasts (Phaff and Starmer, 1987). Surface of leaf, which would be colonized by microbes, are known as phyllospher and main microorganisms that occupy this natural habitat are yeasts (Lindow and Brandi,

2003). The aim of this study is to isolate and investigate production of sorbitol by yeast strain isolated from phyllospher.

MATERIAL AND METHODS

Isolation and Identification of Schizosaccharomyces sp.

For purpose of primary isolation of yeast strains, firstly fig leaves were soaked in the water for 3 days under temperature of 25°C. Then, 0.1ml of the produced solution was inoculated to flask containing isolation medium (40% glucose, 1% yeast concentrate and pH=5) and was then incubated at 30°C in shaker-incubator with velocity of 200rpm (Rao *et al.*, 2004). After 5 days, the solution was inoculated to solid medium and incubated for 2 days; then macroscopic and microscopic morphological properties of colonies were investigated. S*chizosaccharomyyces* sp was identified using biochemical tests (Kurtzman and Fell, 1998). The yeast was maintained on YPD slant media containing 20g/l glucose; 20g/l peptone; 10g/l yeast concentrate and 20g/l agar at 4°C (Altamirano *et al.*, 2000).

Sorbitol Production by Schizosaccharomyces sp.

For Biomass production of *schizosaccharomyces* sp, the yeast was inoculated on flasks containing 20ml medium containing 20g/l glucose; 10g/l yeast extract; 20g/l peptone; 1g/l magnesium sulfate; 2g/l potassium dehydrogenase phosphate and 1g/l ammonium sulfate then was incubated at 28°C in shaker-incubator for 36hrs. Afterwards, through centrifuging the solution in 12000×g for 10mn, yeast cells were precipitated. After 2 times washing the cells by deionization water, their ability for converting sucrose to sorbitol was evaluated. Sorbitol production was conducted by inoculating yeast on 100-ml flasks containing 150g/l sucrose and 10g/l yeast extract (Duvnjak *et al.*, 1991; Sasahara and Izumori, 2005).

Detection of Sorbitol

The fallowing methods were applied for qualitative and quantitative assays of produced sorbitol.

Thin Layer Chromatography (TLC): This method was used for detection of sorbitol in primary steps. For this purpose, 1ml of the medium containing produced sorbitol was placed on silica gel TLC papers and the papers were placed in solution containing propanol, butanol and water to 7:2:1 ratio for 2hrs. Sorbitol solution 10g/l was applied as control sample (Zagustina *et al.*, 2001). Then, the paper was sprayed with colors (sodium hydroxide-potassium permanganate) and after drying, produced sorbitol was evaluated.

Kit: Produced sorbitol by *schizosaccharomyyces* sp was also identified and confirmed using kit)Megazyme, Ireland).

Colorimetric Method: This method includes mild oxidation of sorbitol using sodium metaperiodate under mild acidic conditions (hydrochloric acid) for 10min, then mixing with rhamnose and finally adding Nash reagent (ammonium-acetate 150g/l; acetic acid ml/l and Acetylacetone 2ml/l). The mixture was heated at 53°C in water bath for 15min and after cooling, the amount of produced sorbitol was measured in wavelength of 412nm (Bok and Demain, 1977).

Measurement of Residual Sugar

The residual sucrose which was applied as the carbon source measured by using Anthrone reagent. Briefly, anthrone reagent (0.8 g of *anthrone* was dissolved 50 ml of H2SO4) was added to the fermentation broth, which cells were isolated and sedimented by centrifugation. Then, the product was heated in water bath at 100°C for 10min; afterwards, it was cooled in cold water and recorded the *absorbance* of the solution at the 650 nm (Miller, 1959). Biomass measurement was conducted at the time of production of maximum sorbitol. For this purpose, fermentation medium was centrifuged for 15min in 6000rpm and then was washed two times with distilled water. Then, the medium was dried at 100°C until the weight become (Altamirano *et al.*, 2000).

RESULTS AND DISCUSSION

Isolation and identification of the yeast, was firstly done based on the ability of desired yeast to use sucrose as the carbon source. *Schizosaccharomyces* sp. was isolated from fig leaf and was identified through investigating biochemical tests and also based on morphologic properties. The results from fermentation and absorption tests related to the yeast strain have been presented in table 1.

Table 1: Biochemical tests for the identification of the yeast.

Test type	Type of sugar	*Schizosaccharomyces* sp.
Fermentation	glucose	+
	sucrose	+
	maltose	+
	lactose	-
Absorption	glucose	+
	sucrose	+
	maltose	+
	lactose	-
	malibose	-
	D-xylose	-
	L-arabinose	-
	D-mannitol	-
	inulin	-
	inositol	-
	starch	-
	citrate	-

The macroscopic and microscopic properties of colonies are shown in figures 1 and 2. *Schizosaccharomyces* sp. have soft, flat and white to beige color colonies on PDA medium.

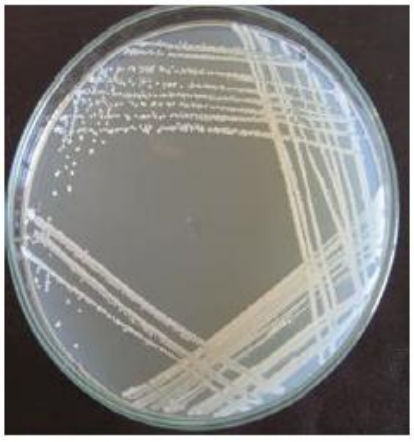

Figure 1: Colony morphology of *schizosaccharomyces* sp. in PDA medium

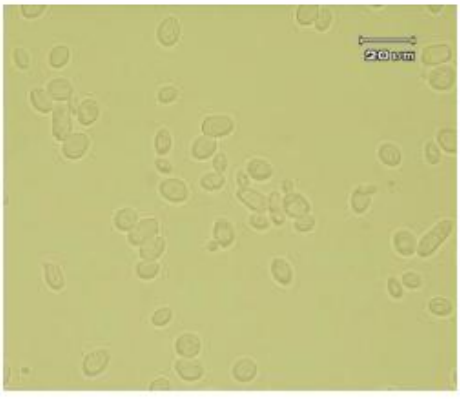

Figure 2: Microscopic profile of cells of *schizosaccharomyces* sp.

Qualitative and Quantitative Evaluation of Produced Sorbitol

Schizosaccharomyces sp. was able to produce sorbitol from sucrose after 24hrs. The maximum yield of sorbitol was obtained on the fourth day. Produced sorbitol was assayed with TLC (figure 3). Also, produced sorbitol by the desired yeast was identified and confirmed using kit. The levels of remaining sucrose and biomass during the process is illustrated in figure 4.

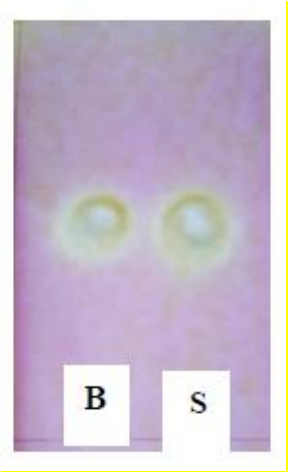

Figure 3: Assay of produced sorbitol by TLC (B: control and S: produced sorbitol)

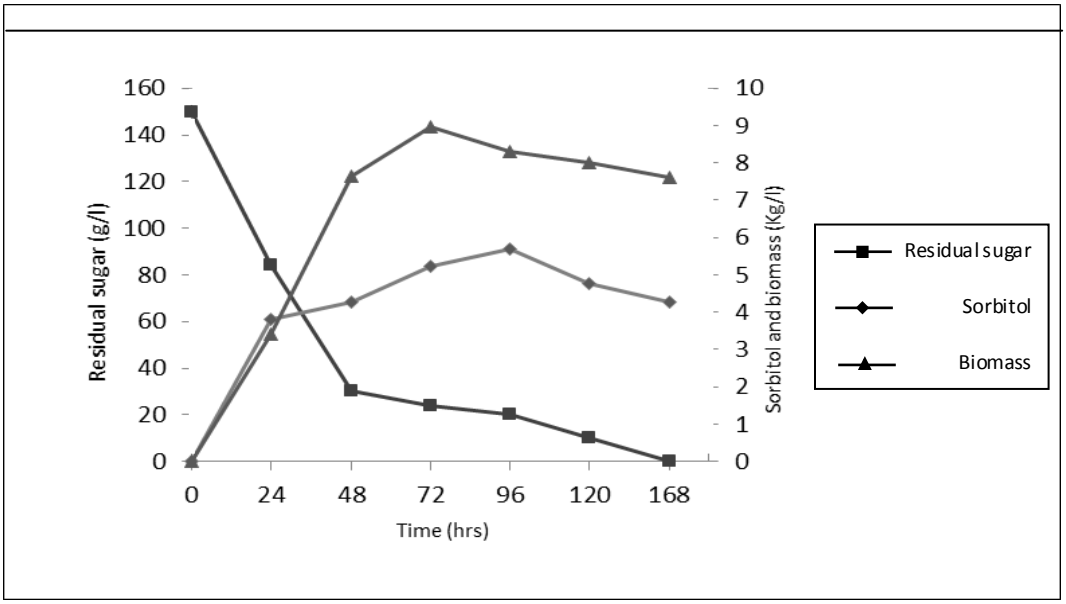

Figure 4: Sorbitol production curve by *schizosaccharomyces* sp.

At the present study, *schizosaccharomyces* sp. isolated from phyllospher has been able to produce sorbitol from sucrose with high efficiency. Based on our knowledge, there has been no similar study in regard with producing sorbitol by phyllospher yeasts so far, Most studies have been conducted on *Candida boidinii*. Vongsuvanlert and Tani purified and isolated xylose isomerase enzyme, which is involved in sorbitol production from glucose, from this yeast (vogsuvanlert and Tanni, 1988).

Sasahara and Izumori isolated *Aureobasidium Pullulans* LP23 strain from soy sauce mash in medium containing 1% L-fructose and 0.1% D-glucose. This strain was able to produce L-sorbitol from L-fructose. The results indicated that adding erythritol to the mixture can accelerate production process (Sasahara and Izumori, 2005).

Another microorganism, on which the most studies have been conducted, is *Zymomonas mobilis*. In a study, Viikari studied the formation of levan and sorbitol from sucrose by *Zymomonas Mobilis*. The results also indicated that the efficiency of levan and sorbitol production is respectively equal to 8% and 11% in a medium containing 150g/l sucrose (Viikari, 1984a). Sookkheo *et al.*, have also studied sorbitol production from sucrose in *Zymomonas mobilis* strain. The study indicated that the highest yield of sorbitol is attained in these conditions: 250g/dm^3 sucrose, pH=7.5 at 30°C (Sookkheo *et al.*, 1991). Ro and Kim have also studied the biological conversion of sucrose to sorbitol using *Zymomonas mobilis* treated with toluene and invertase (Ro and Kim, 1991). Barros *et al.*, studied the effect of ultrasonic waves on produced sorbitol by *Zymomonas mobilis* in high sucrose concentration (Barros *et al.*, 2006). At the present study, for the first time a strain of yeast has been isolated in Iran, which is able to produce 5.71g.l sorbitol in medium containing 150g/l sucrose.

Sorbitol is a polyol has abundant applications and as the results of the present study indicate, sorbitol can be produced using yeasts existed in phyllospher. Different factors that can affect amount of sorbitol production included, increase in concentration of primary sugar and the yeast biomass. Another effective factor is aeration, low levels of oxygen is necessary for better growth of the yeast and sorbitol production (Petrovska *et al.*, 2000). Isolation of sorbitol producing yeast strains and optimization environmental conditions in future can lead to use of yeasts for biological sorbitol production in larger scales.

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