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β-Glucan production of *Saccharomyces cerevisiae* by using malva nut juice production wastewater

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Abstract

Three yeast strains such as *S. cerevisiae* Angel®, *S. cerevisiae* TISTR 5020 and *S. cerevisiae* TISTR 5919 isolates were used in this study. β-Glucan production and the yield of three strains of *S. cerevisiae* by using malva nut juice production wastewater were studied in order to reduce environmental pollution. The results showed that significantly higher %yield and β-glucan production was founded in *S. cerevisiae* TISTR 5919 (0.67 ± 0.01%w/v and 15.01%w/w; respectively) than that *S. cerevisiae* Angel® and *S. cerevisiae* TISTR 5020 (0.39±0.01%w/v, 12.69±2.62%w/w and 0.41±0.03%w/v, 8.33±1.26%w/w; respectively). Consequently, *S. cerevisiae* TISTR 5919 was selected to compare cell morphology by scanning electron microscopy (SEM) and the thickness of the cell wall by transmission electron microscopy (TEM) between cultured in YPD medium (control) and malva nut juice production wastewater. Our results found that multiple budding cell, thinner cell wall and abnormal cell were exhibited when yeast was cultivated in YPD medium supplement with malva nut juice production wastewater. Overall, the obtained results implied that the wastewater from malva nut juice production could be activate budding process and β-glucan formation, effected to the cell wall thinner and reduced the cell dry weight of yeast cell.

Introduction

β-Glucans are group of polysaccharides that are composed of glucose units linked together with β-glycosidic bonds (Klis *et al*., 2002). They have been used in many industries, such as the pharmaceutical, food, feed, and cosmetics industries (Reed and Nagodawithana, 1991). β-Glucans also exhibit medicinal properties such as antitumor, antimicrobial and antioxidant activities plus mycotoxin absorption (Ross *et al*., 1999; Chen and Seviour, 2007; Magnani *et al*., 2011) as well as uses in stimulation of the immune response in animals, such as shrimps, weaned pigs and mice, and the reduction of blood cholesterol and glucose levels (Ostroff, 1997; Nicolosi *et al*., 1999; Hayen and Pollmann, 2001; Ortuno *et al*., 2002).

Generally, cell wall of *Saccharomyces cerevisiae,* being about 70 nm thick, accounts for 20% of the cell's weight (Walker, 1999). The composition of the cell wall is mainly mannoproteins, β-glucan (85-90% of cell dry mass), small amount of chitin (1-3%) and lipid (2-5%) (Klis, 1994). Thus, *S. cerevisiae* has been considered as an interesting source of β-glucan production since the β-glucan from *S. cerevisiae* has various properties that are more preferable to those found in other sources (Nguyen *et al*., 1998). The β-glucan has been made up about 55 - 65% w/w of yeast cell walls, consisting of both long chains of β-1,3-glucan (about 85% of the total) and short chains of β-1,6-glucan (Klis *et al.*, 2002). *S. cerevisiae* can be rapidly and easily grown in a diverse array of culture media at a low production cost and its whole genome is already known. Thus, *S. cerevisiae* is a good natural choice for β-glucan production. However, in terms of improving β-glucan production from microbial cells there appears to be little, and no specific information on the effect of wastewater on b-glucan production in *S. cerevisiae* compared with other organisms. For example, *Botryosphaeria rhodina* was stimulated to produce β-glucan by the use of emulsified media (Crognale *et al*., 2007), while induction of β-glucan synthetase in mushrooms was attained by culturing them in olive mill wastewater (Reverberi *et al.*, 2004). Several factors including the type of culture medium, carbon source, pH, temperature, aeration rate and culturing conditions influence the morphology and composition of the cell wall during growth process. β-Glucan production by *S. cerevisiae* in a batch fermenter was found to be optimal when

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grown in Yeast Peptone Dextrose (YPD) pH 4.0, with galactose as the carbon source, at 37°C and well-aerated conditions of pO_2 > 50% saturation (Aguilar-Uscanga and Francois, 2003). However, to the best of our knowledge no information about the effect of malva nut juice production wastewater on the cell dry weight and β-glucan production. Therefore, in this study, we investigated the effect of malva nut wastewater on %yield (cell dry weight) and β-glucan production. Then the best strain was selected to compare cell morphology and the thickness of the cell wall when yeast was cultivated in YPD media (control) and the media supplemented with malva nut juice production wastewater.

Materials and Methods

Yeast cultivation

Three yeast strains were used in this study such as *S. cerevisiae* Angel®, *S. cerevisiae* TISTR 5020 and *S. cerevisiae* TISTR 5919. *S. cerevisiae* Angel® was gifted from Food Microbiology laboratory, Food Technology department, Chulalongkorn university. *S. cerevisiae* TISTR 5020 and *S. cerevisiae* TISTR 5919 were purchased from Microbiological resources center,Thailand Institute of Scientific and Technological Research, Thailand. Starter culture (5 ml) was inoculated into flask containing 500 ml malva nut juice production wastewater supplemented with 1%w/v yeast extract, 2%w/v peptone and 2%w/v dextrose (Himedia, India)). Cells were incubated at 30°C, pH 4 in reciprocal shaking 200 rpm for 48 h. The yeast cultures were collected by using centrifugation at 8000 x g for 10 min, freeze dried and determining the %yield of yeast cell dry weight and β-glucan contents.

Glucan contents determination

β-Glucan contents in yeast powder were analyzed using a Yeast Beta-Glucan Assay Kit (Megazyme, Ireland) as follows. For total-glucan content, 100 mg of milled yeasts were placed in test tube then 1.5 ml of 37% hydrochloric acid was added. The solution was mixed and incubated at 30°C for 45 min (mixed every 15 min). Then, 10 ml of distilled water was added, mixed and incubated at 100°C for 2 h before added with 10 ml of 2 N potassium hydroxide(KOH). The solution was taken, adjusted volume to 100 ml with sodium acetate buffer pH 5 and mixed. After that, the mixtures were centrifuged at 1,500 x g for 10 min. Samples (100 µl) were taken to each test tube before added with 100 µl of a mixture of exo-1,3-β-glucanase plus β-glucosidase and then incubated at 40°C for 60 min. Finally, 3 ml of glucose oxidase/peroxidase were added and incubated at 40°C for 20 min. The absorbance was measured at 510 nm with spectrophotometer (Celli, CE1011, England). The concentration of glucose in the sample was calculated from the assay kit procedure.

For β-glucan content, 100 mg of milled yeasts were placed in test tubes and then 2 M potassium hydroxide(KOH) (2 ml) was added. The pellets were stirred with magnetic stirrer in ice bath for 20 min. Next, 8 ml of 1.2 M sodium acetate buffer (pH 3.8) were added to the mixture. Then, amyloglucosidase plus invertase (200 µl) were added, incubated at 40°C for 30 min and mixed by vertex stirrer. After that, the mixture was centrifuged at 1,500 x g for 10 min. Supernatant (100 µl) were taken to test tube. Glucose oxidase / peroxidase (3 ml) were added to each tube and incubated at 40°C for 20 min. The absorbance was measured at 510 nm with spectrophotometer (Celli, CE1011, England). The concentration of glucose in the sample was calculated from the assay kit procedure. For the amount of b-glucan content, it was calculated by total-glucan substract β-glucan (Mongkontanawat *et al.*, 2011).

Yeast cell morphology and the thickness of cell wall

The best strain was selected and cultured in YPD medium and malva nut juice production wastewater supplemented with 1%w/v yeast extract, 2%w/v peptone and 2%w/v dextrose (Himedia, India)), adjusted to pH 4.0, incubated at 30oC with shaking at 200 rpm for 24 h. Yeast cells were then sampled from the culture and photographed with a scanning electron microscopy (SEM) (JEOL, model JSM-5410LV, Japan) at a magnification of 10,000 X was used to capture images of whole cell shape and cell wall surface of yeasts grown under the studied culture conditions. For the thickness of cell wall, yeast cells were prepared by negative staining method and photographed with a transmission electron microscopy (TEM) (JEM-2100, Japan) was used to capture images of yeast cell wall of yeasts grown under the studied culture conditions. Finally, the yeast cultures were collected, freeze dried and determining the %yield of yeast cell dry weight and β-glucan contents of yeasts grown under the studied cultivate conditions.

Statistical analysis

The data were collected from triplicates. Analysis was performed by computer software. All of data were analyzed with Analysis of Variance (ANOVA) and multiple comparison were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

β-Glucan production

From table 1, high level of total-glucan content (15.38±3.00%w/w) when yeast were cultivated in media supplemented with malva nut juice production wastewater was found in *S.cerevisiae* TISTR 5919. However, not significantly different (p≤ 0.05) from *S.cerevisiae* Angel® (13.27±2.57 %w/w), In contrast, still significantly different (p≤ 0.05) from S.cerevisiae TISTR 5020. High level of α-glucan content was exhibited in *S.cerevisiae* Angel® (0.58 ±0.07%w/w) when yeast were cultured in media supplemented with malva nut juice production wastewater and significantly different (p≤ 0.05) from *S.cerevisiae* TISTR 5020 and *S.cerevisiae* TISTR 5919 (0.38 ±0.08 and 0.37±0.08%w/w, respectively). For β-glucan content, our result was found that β-glucan contents of *S.cerevisiae* TISTR 5919 (15.01±1.94%w/w) higher than other species, however, not significantly different from *S.cerevisiae* Angel® (12.69±2.62%w/w). In addition, the significantly lowest β-glucan content was found in *S.cerevisiae* TISTR 5020 (8.33±1.26%w/w).

For the yield of cell dry weight, significantly (p≤ 0.05) highest yield was also exhibited in *S.cerevisiae* TISTR 5919 (0.67 ±0.01%w/v) when yeast was cultivated in media supplemented with malva nut juice production wastewater. The low level of yield of cell dry weight when yeast were cultured in media supplemented with malva nut juice wastewater was exhibited in *S.cerevisiae* TISTR 5020 and *S. cerevisiae* Angel® (0.41 ±0.03 and 0.39 ±0.01%w/v, respectively) (Figure 1). Based on high β-glucan content and the yield of cell dry weight, therefore *S.cerevisiae* TISTR 5919 was selected to further monitored to compare the cell morphology and the thickness of the cell wall when yeast was cultured in YPD medium (control) and the medium supplemented with malva nut juice production wastewater.

Yeast cell morphology and the thickness of cell wall

Our result found that, the result of scanning electron microscopy showed that the highest number of bud scars was exhibited when yeast was cultivated with malva nut juice production wastewater supplemented with 1%w/v yeast extract, 2%w/v peptone and 2%w/v dextrose, adjusted to pH 4.0 (Figure 2). This effect could be explained that phenolic compound in the malva nut juice production wastewater could activate FKS1 which are the gene that activate actin cytoskeleton in budding process (Delley and Hall, 1999). Consequently, the budding process and β-glucan content were increased (as well as found in this study). Cell wall is biosynthesized mostly during budding process (Cabib, 2001). Kawamura *et al*. (2011) reported that malva nut bark have % total phenol 43.7± 6.08% and phenolic compound 4.82 mg/ml in malva nut juice was founded in this study. It clearly showed that β-glucan content in yeast cultivated in media supplemented with malva nut juice production wastewater slightly significantly (p≤ 0.05) higher than control (Table 2). In the other hand, yield of cell dry weight significantly (p≤ 0.05) lower than control. Furthermore, the slender cell wall and abnormal cells were demonstrated when yeast was cultured in a medium supplemented with malva nut juice production wastewater (Figure 3). In conclusion, when yeast cultured in the wastewater which could significantly enhance the β-glucan production. However, the cell wall could be thinner than the control condition. Thus, it is clear that malva

Table 1. Total-glucan, α-glucan and β-glucan content (as %w/w dry weight) of the cell of the *S. cerevisiae* Angel®, *S. cerevisiae* TISTR 5020 and *S. cerevisiae* TISTR 5919 isolates* when cultured in YPD media supplemented with malva nut juice production wastewater for 48 h.

Strains	Total-glucan (%w/w)	α-glucan (%w/w)	β-glucan (%w/w) **
S.cerevisiae Angel®	13.27±2.57 [a]	0.58±0.07 [a]	12.69±2.62[a]
S.cerevisiae TISTR 5020	8.71±1.26 [b]	0.38±0.08 [b]	8.33±1.26[b]
S.cerevisiae TISTR 5919	15.38±3.00 [a]	0.37±0.08 [b]	15.01±1.94[a]

*Data are shown as mean±one SD and are derived from three replicates. **Means within a column followed by a different letter are significantly different (p≤0.05)

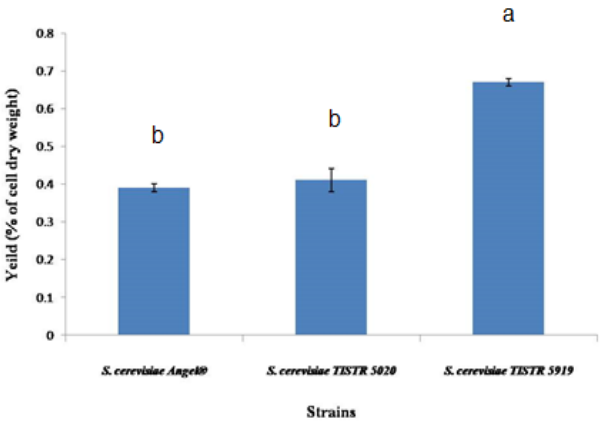

Figure 1. Yield of cell dry weight of the *S. cerevisiae* Angel®, *S. cerevisiae* TISTR 5020 and *S. cerevisiae* TISTR 5919 isolates when cultured in YPD media supplemented with Malva nut juice production wastewater for 48 h.

Table 2. Yield of cell dry weight (%w/v) and the β-glucan content (as %w/w dry weight) of the *S. cerevisiae* TISTR 5919 isolate when cultured in YPD media (control) or that supplemented with Malva nut juice production wastewater when cultured for 24 h

Treatments	Yield (%of cell dry weight)	β-glucan content (%w/w of cell)
Control	0.28 ±0.05[a]	8.02 ±0.50[b]
Malva nut juice production wastewater	0.12±0.02[b]	8.58±0.50[a]

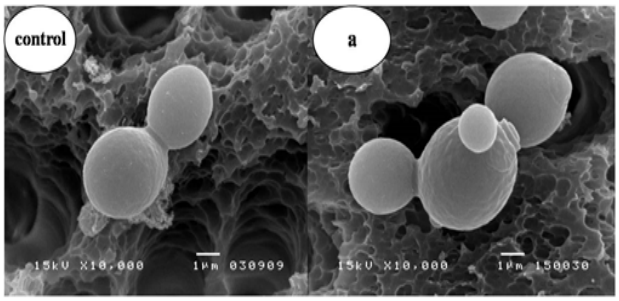

Figure 2. Representative SEM micrographs(10,000 xmagnification) of the *S. cerevisiae* TISTR 5919 isolate when cultured in YPD media (control) or that supplemented with wastewater from malva nut juice production(a)

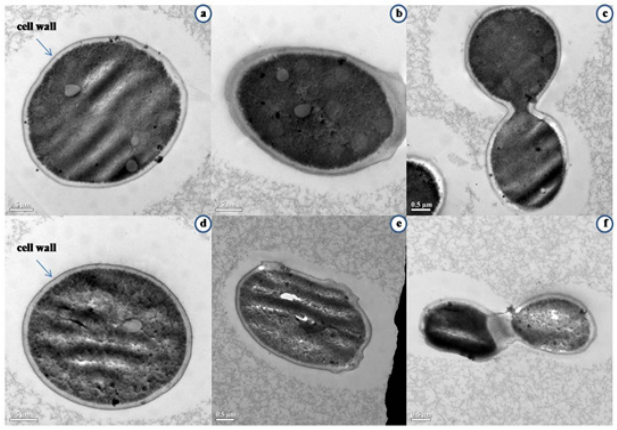

Figure 3. Representative TEM micrographs of the *S. cerevisiae* TISTR 5919 isolate when cultured in YPD media (a-c) or that supplemented with wastewater from malva nut juice production(d-f)

nut juice production wastewater could only activate β-glucan formation of yeast cell, which could be explained that yeast under stress conditions tried to produce larger amount of cell wall component to protect cell from stress. Therefore, β-glucan synthase is redistributed in response to cell wall stress to repair general cell wall damage (Delley and Hall, 1999; Klis *et al.*, 2002). The result corresponded well with the previous studies which reported that malva nut alcohol extract inhibited the gram positive pathogenic bacteria such as *Staphylococcus aureus* and *Bacillus cereus* (Pukahuta *et al.*, 2006). Moreover, our currently research revealed that malva nut juice also inhibited *Lactobacillus casei* TISTR 390.

Conclusion

High β-glucan production and yield of cell weight was exhibited in *S. cerevisiae* TISTR 5919 isolates when yeast was cultured in a medium supplemented with malva nut juice production wastewater. Moreover, in this treatment, significant change in cell morphology was found, which cells possessed a high number of bud scars, abnormal cell and thinner cell wall compared with control condition. In conclusion, these results indicated that malva nut juice production wastewater could activated budding process and β-glucan formation, effected to the cell wall thinner and reduced the cell dry weight of yeast cell. This research could be the new way to reduce the waste form the malva nut juice factory and obtain the new medium for β-glucan production form yeast.

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