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Development of β-glucan production from microorganisms as active ingredients in cosmeceutical products for skin youthfulness

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ABSTRACT: This study aims to select the potential microorganisms that contain high beta-glucan (β-glucan) and develop its cultivation, extraction, and purification methods from the selected microbe. The derived β-glucan would be tested for its toxicity; the superoxide dismutase (SOD) activity as well as the production of collagen and elastin for development as an ingredient in the cosmetic industry would also be examined. Under this project, *Saccharomyces cerevisiae* TISTR 5027 is the supreme strain for β-glucan production from 9 selected *S. cerevisiae* strains. The best medium for culturing *S. cerevisiae* TISTR 5027 is yeast mold broth (YM), supplemented with 0.5% peptone, agitated at 250–300 rpm, and incubated at 30–37 °C for 60–72 h. After extraction, the derived β-glucan at various concentrations was subjected to a toxicity test against human keratinocyte (HaCaT) cells and normal human dermal fibroblast (NHDF) cells. No adverse effect of cells was observed with the concentrations of β-glucan of 3.13, 6.25, 12.50, 25.00, 50.00, and 100.00 μg/ml. Interestingly, the concentrations of β-glucan of 12.50 and 25.00 μg/ml could activate the production of collagen in NHDF cells at 3.22 ± 0.08, and 3.91 ± 0.17%, respectively, while the production of collagen in untreated NHDF cells was 2.82 ± 0.13%. Additionally, the same amounts of β-glucan could enhance the production of elastin in NHDF cells at 86.12 ± 1.62, and 84.11 ± 6.02%, respectively, which were higher than those in untreated NHDF cells (78.71 ± 3.60%).

KEYWORDS: β-glucan, superoxide dismutase, collagen, elastin, *Saccharomyces cerevisiae*

INTRODUCTION

β-Glucan is a polysaccharide of D-glucose monomers linked by β-glycosidic bonds. Several studies have indicated that yeast, fungi, bacteria, algae, and plants are the main sources of β-glucan [1]. According to Klis et al [2], the cell wall of the typical yeast occupies approximately 15–30% of the cell dry weight. It mostly consists of mannoprotein (35–40%), fibrous β-1,3-glucan as well as branched β-1,6-glucan (50–55%), chitin (1–2%), and lipid (1–3%). Although yeast is primarily considered the best source of β-glucan, due to the amount and types of β-glucans [3], the characteristics and contents of β-glucans obtained from yeast vary by species [4]. Alfonso et al [5] studied the wild yeast from numerous fruits collected in a Mexico forest and found that the compositions and amounts of β-glucan derived from 44 isolates of these yeast were diverse due to their species and geography. The exceptional strains reported for β-glucan production are *S. cerevisiae* YLG36 and *S. cerevisiae* YPG43. The amount of β-glucan obtained was 1.67 μg/mg dry cell weight, whereas those of the other species were only in the range of 0.52–1.20 μg/mg dry cell weight [5]. Not only the amount of β-glucan accumulated in the cells but also the knowledge of fermentation processes led to the use of *S. cerevisiae* as a potential source for β-glucan production [6, 7].

At present, β-glucan is used in the pharmaceutical, cosmetic, food, and feed industries, etc. [8, 9] because it stimulates body immune responses, thus reducing cancers and diabetes [10], lowering blood cholesterol [11], and inhibiting cell inflammation [12]. Concerning cosmetics, β-glucan can enhance skin immunity through the Langerhans cells located in the epithelium of mucosal tissues and epidermis. It also boosts the production of collagen, elastin, and hyaluronic acid through Pattern recognition receptors (PRRs) located on the Langerhans cells [13]. The crosslinks of these compounds are highly beneficial to the vitality of skin cells [14]. Additionally, β-glucan has shown significant effects on skin cells as an antioxidant [15] and a moisturizing agent [16]. Based on the results from these studies, β-glucan is widely used as the main ingredient in many skin care products.

This study aims to screen and optimize the growth conditions of the potential yeast strains that contain the highest amount of β-glucan in the cell wall. It also attempts to extract soluble β-glucan using the alkaline-acidic isolation methods and evaluate the efficacy of the extracted β-glucan vis-a-vis the skin cells. The

knowledge gained from this study can be used for the development of β-glucan as an ingredient for cosmeceutical products such as acne cream or other skin care products in the Thai cosmetic industry in the future.

MATERIALS AND METHODS

Yeast strains

Yeast strains that are easy to grow and have high growth rates, *viz*., 9 strains of *S. cerevisiae*: TISTR 5027, TISTR 5046, TISTR 5954, TISTR 5955, TISTR 5039, TISTR 5868, TISTR 5916, TISTR 5343, and TISTR 5896 from the Thailand Institute of Scientific and Technological Research Culture Collection (TISTR Culture Collection) were selected for β-glucan production in this study.

Measurements of yeast growth rates

Growth curves of 9 yeast strains were determined by the method adopted by Jung et al [17]. The 0.1 ml of overnight culture at a cell concentration of about 10^8 cells/ml was transferred to a 96-well plate containing 0.9 ml of yeast mold broth (YM broth) (Difco™, Sparks, MD, USA) with the following composition (per liter): malt extract 3 g, yeast extract 3 g, peptone 5 g, and dextrose 10 g. The plate was incubated at 30 °C for 24 h. The optical densities at 600 nm of every yeast strain were measured hourly and recorded by a microplate reader. Thereafter, the top 5 strains showing the highest optical density were selected for determining the β-glucan contents in the next experiment.

Determination of β-glucan contents

The 5 selected yeast strains were cultured in 100 ml of YM broth at 30 °C. The cells were harvested by centrifugation at 5,000–7,000 rpm when they reached their stationary phase, as a high amount of β-glucan has been previously reported during this stage of yeast cells [5]. The collected cells were extracted through the acid and alkali extraction processes [18]. The β-glucans obtained were dried by a freezer dryer. The amounts of β-glucan were analyzed and determined as prescribed by Gründemann et al [19] using a mushroom and yeast β-glucan assay kit (K-YBGL) (Megazyme, Wicklow, Ireland). The contents of the extracted β-glucan were calculated based on the amount of D-glucose by all β-glucans in the sample by a "difference" method; total glucan = (α + β glucan) was measured by acid-mediated degradation while α-glucan was selectively measured by enzymatic degradation and subtracted to yield β-glucan through the following equation:

$$\beta\text{-glucan} = \text{total glucan} - \alpha\text{-glucan}$$

Evaluating the effect of different culture media on yeast cell dry weight

As the amount of β-glucan depends on the amount of yeast cell dry weight obtained, the modified YM medium, supplemented with carbon and nitrogen from different sources such as glucose, yeast extract, peptone, and ammonium sulfate, was used to enhance the effects of the culture media on yeast cell dry weight. In this study, the 5 formulations of media, *viz*., YM (10 g, Difco™) + glucose (15 g, Kemaus, Cherrybrook, Australia), YM (10 g) + yeast extract (10 g, Difco™), YM (10 g) + peptone (10 g, Difco™), YM (10 g) + ammonium sulfate (5 g, Ajax Finechem, Scoresby, Australia) and YM broth were prepared and used for culturing the selected yeast strain containing the highest amount of β-glucans. The selected yeast strain was cultured in 100 ml of each medium, using a 500 ml flask. The culture was incubated at 30 °C with agitation speed at 200 rpm for 48 h. Thereafter, the cells were centrifuged at 5,000–7,000 rpm for 10 min and dried using a hot air oven at 65 °C for 24 h until a constant weight was achieved. The dried cells derived from each medium were weighed and compared.

Evaluating the effects of different fermentation parameters on β-glucan production

The selected yeast strain from the previous study was cultured in a 5 l fermenter by using a 4 l medium with the most significant effects on increasing the yeast cell dry weight. The 10% (v/v) of overnight culture at a cell concentration of 10^8 cells/ml was used as an inoculum size. The batch fermentation was operated at 30 °C and 37 °C with an airflow rate of 1 vvm and agitation speed varying from 150 rpm to 300 rpm for 72 h. Thereafter, the cells from each condition were harvested by centrifugation at 5,000–7,000 rpm for 10 min and dried by freezer dryer. β-glucans were homogenized, extracted, and dried by the method mentioned previously. The amounts of β-glucans obtained were determined and compared.

Skin cytotoxicity of extracted β-glucan

The cytotoxic effects of the extracted β-glucans were examined following the method by Yowaphui [20], using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt (WST-1) (Abcam, Cambridge, England) and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) (Thermo fisher scientific, Waltham, USA) against human keratinocyte (HaCaT) cell and normal human dermal fibroblast cell (NHDF), respectively. Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific) on 96-well plates under 5% carbon dioxide at 37 °C for 24 h to obtain a cell density of 1×10^5 cells/ml. In each assay, the cells were challenged with various concentrations of extracted β-glucans which were 3.13, 6.25, 12.50, 25.00, 50.00, 100.00, and 200.00 μg/ml. The cells in both plates were further incubated under 5% carbon dioxide (CO_2) at 37 °C for 72 h before washing once with phosphate buffered

saline (PBS) (Sigma-Aldrich, Saint Louis, USA). Then, 100 µl of WST-1 solution and DMEM were added to the plate containing HaCaT cells in the ratio of 1:10 and incubated at 37 °C for 30 min, whereas 100 µl of MTT solution and DMEM were added to the plate containing NHDF cells in the ratio of 1:10 and incubated at 37 °C for 3 h. The optical density (OD) at 450 nm of the cell mixture was assessed and used for determining the HaCaT cell viability while OD at 572 nm was used for measuring the NHDF cell viability. The percentages of HaCaT and NHDF cell viabilities were calculated by the following equation:

$$\% \text{ Cell viability} = \frac{\text{optical density of sample} \times 100}{\text{optical density of control sample}}$$

Assay of superoxide dismutase (SOD) activity elicited by extracted β-glucan

As the ability to reduce aging processes is one of the major requirements of a cosmetic ingredient, the roles of the extracted β-glucans in SOD activity were determined in this study because this enzyme acts as a powerful antioxidant and helps in removing the free radicals produced during aging processes. The HaCaT cells cultured in DMEM with a cell density of 1×10^5 cells/ml were prepared and used. The extracted β-glucans at concentrations of 25.00, 50.00, and 100.00 µg/ml were applied to the cells grown on the plate. The 10 µg/ml of standard SOD (Sigma-Aldrich) was used as a positive control. This experiment was performed using a SOD assay kit. The cells were treated as per the method described in the kit. The OD at 450 nm of the supernatant derived from treated cells was used for measuring and calculating the activities of the SOD generated by extracted β-glucans. The amounts of supernatant proteins were measured by using a bicinchoninic acid (BCA) assay kit (Pierce, Rockford, USA). The method for calculation of viability was followed by the ISO 10993-5 [21] test for *in vitro* cytotoxicity and guidelines for cell viability assays. The method for the calculation of SOD was as per the directions in the assay kit. The OD at 450 nm of the supernatant derived from the treated cells was used for measuring and calculating the activities of the SOD generated by the extracted β-glucans. As SOD activities need to be compared with an equal amount of protein content, the amounts of supernatant proteins were measured by using the BCA assay kit. The protein contents were measured and calculated through the OD at 562 nm.

Assessment of cellular collagen and elastin production by extracted β-glucans

The efficiency of the extracted β-glucans as a cosmetic ingredient was determined through the production of cellular collagen and elastin activated by the extracted β-glucans. A collagen assay kit (Cosmo Bio CO., Ltd., Tokyo, Japan) was used for the detection of human collagen type I as per the method described by Young et al [22]. A Fastin™ elastin assay kit (Biocolor Ltd., Carrickfergus, England) was used for the detection of cellular elastin. However, cellular elastin needed to be extracted by the method adopted by Naito et al [23] before performing the assay. The NHDF cells cultured in DMEM with a cell density of 3×10^4 cells/ml were used for both assays. The extracted β-glucans at concentrations of 6.25, 12.50, and 25.00 µg/ml were applied to the cells grown on the plate. We selected the safety dose that showed the viability of NHDF cells at more than 70%. The 25 µg/ml of ascorbic acid (Loba Chemie Pvt. Ltd., Mumbai, India) was used as a positive control. The cells were treated as per the method provided in each kit. OD at 450 nm of treated cells was used for measuring and calculating the amount of human collagen type I and OD at 517 nm for measuring and calculating the amount of the cellular elastin.

Statistical analysis

All experiments in this study were performed thrice, and the results were shown as mean ± standard deviation. The data series were tested for homogeneity of variance using Levene's test and for normality using the Kolmogorov-Smirnov test. Differences among experimental treatments were tested by one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test as a post-hoc test. Values were considered statistically different at a 95% confidence level ($p < 0.05$), using the SPSS program (Version 23.0).

RESULTS

As shown in Fig. 1, all yeast strains grown in YM broth at 30 °C for 24 h entered a stationary phase within 7–9 h. The values of OD at 600 nm of the 9 yeast strains were in the range of 0.7–1.0. TISTR 5027, TISTR 5896, TISTR 5916, TISTR 5955, and TISTR 5954 were the top 5 *S. cerevisiae* strains showing the highest growth rates at 0.308, 0.282, 0.273, 0.228, and 0.323 h^{-1}, respectively. There was no statistically significant difference among these strains ($p > 0.05$).

However, in terms of β-glucan contents, these top 5 strains showed statistically significant inter se differences ($p < 0.05$). The amounts of β-glucan obtained from each strain ranged from 11.74 to 40.60 mg/100 mg of yeast cell dry weight (w/w). As shown in Fig. 2, The highest amount of β-glucan was obtained from *S. cerevisiae* TISTR 5027 while the lowest was from *S. cerevisiae* TISTR 5954. Therefore, *S. cerevisiae* TISTR 5027 was selected as a potential strain for β-glucan production throughout this study.

The effects of the 5 formulations of media on the dry cell weight of *S. cerevisiae* TISTR 5027 are shown in Fig. 3. Following the statistical analysis, the dry cell weight of *S. cerevisiae* TISTR 5027increased signif-

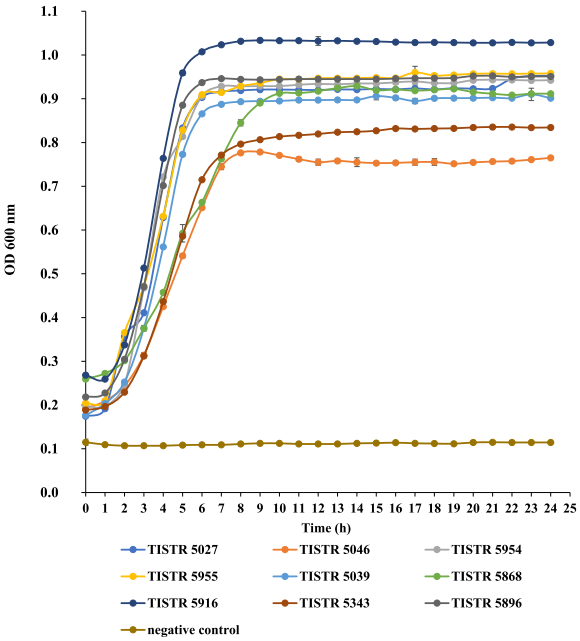

Fig. 1 Growth curves of the 9 selected yeast strains grown in YM broth at 30 °C for 24 h. Results were expressed in mean ± SD, where $n = 3$. No significant differences for each treatment were determined by one-way ANOVA and Tukey's post-hoc tests ($p > 0.05$).

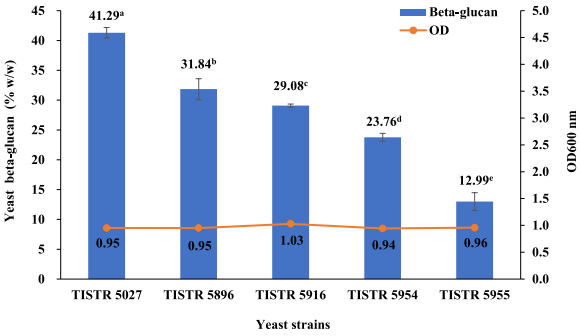

Fig. 2 The amounts of β-glucan obtained from the top 5 strains and showing the highest OD at 600 nm. Results were expressed in mean ± SD, where $n = 3$. Significant differences for each treatment were determined by one-way ANOVA and Tukey's post-hoc tests ($p < 0.05$).

icantly when either YM + peptone, YM + $(NH_4)_2SO_4$, or YM + glucose was used as culture media individually, compared to those grown in YM broth (Control medium) ($p < 0.05$) alone. The highest yield of 0.82 ± 0.15 g/100 ml of *S. cerevisiae* TISTR 5027 dry cell weight was harvested from a modified medium YM + peptone, accounting for approximately double time in yield increasing from YM broth alone. While YM + peptone enhanced yield over that of the control medium, the addition of another nitrogen source to

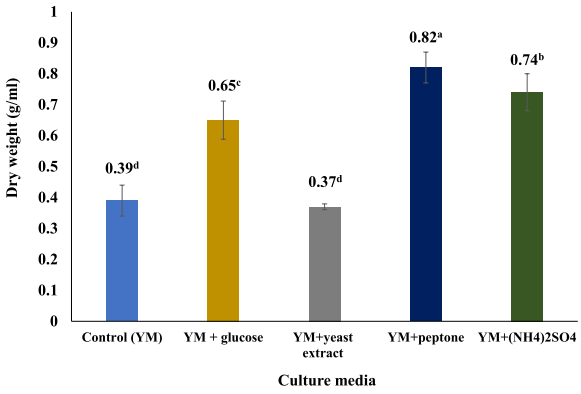

Fig. 3 The dry cell weights of *S. cerevisiae* TISTR 5027 derived from culturing in 5 media, *viz.*, YM + glucose, YM + yeast extract, YM + peptone, YM + $(NH_4)_2SO_4$, and YM broth at 30 °C for 48 h. Results were expressed in mean ± SD, where $n = 3$. Significant differences for each treatment were determined by one-way ANOVA and Tukey's post-hoc tests ($p < 0.05$).

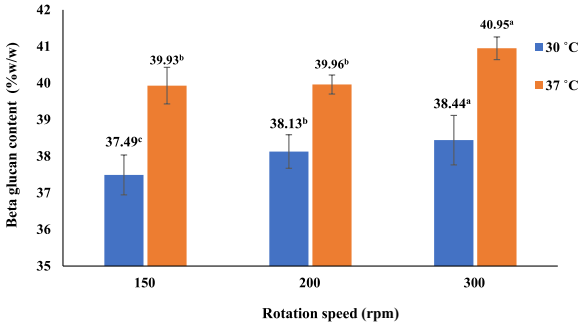

Fig. 4 Comparisons of β-glucan contents of *S. cerevisiae* TISTR 5027 grown in YM + peptone for 72 h with different temperatures and rotational speeds. Results were expressed in mean ± SD, where $n = 3$. No significant differences were determined for each treatment by one-way ANOVA and Tukey's post-hoc tests ($p > 0.05$).

this modified medium (YM + $(NH_4)_2SO_4$) led to a lower dry cell weight yield of 0.74 ± 0.06 g/100 ml. Surprisingly, the modified medium YM + yeast extract did not boost the yield of *S. cerevisiae* TISTR 5027 dry weights. Among the 3 promising modified media, YM + glucose medium gave the lowest yield of 0.65 ± 0.06 g/100 ml. In addition, the possible roles of fermenter parameters including temperatures and rotational speeds on *S. cerevisiae* TISTR 5027 grown in YM + peptone medium in a 5-liter fermenter will be further investigated.

β-Glucan contents of *S. cerevisiae* TISTR 5027 grown in YM + peptone for 72 h with different temperatures and rotational speed or aeration rate are reported in Fig. 4. The amounts of β-glucan of 37.50 ± 0.54% w/w, 38.13 ± 0.46% w/w, and

38.45 ± 0.68% w/w were obtained from cells grown at 30 °C using the rotational speeds of 150, 200, and 300 rpm, respectively. In addition, the amounts of β-glucan of 39.93 ± 0.50% w/w, 39.97 ± 0.26% w/w, and 40.96 ± 0.31% w/w were obtained from cells grown at 37 °C using the rotational speeds of 150, 200, and 300 rpm, respectively. After statistical data analysis, no significant difference was observed among these conditions ($p > 0.05$), suggesting that the aeration rate did not play an important role in enhancing the β-glucan contents. Interestingly, the amounts of β-glucans obtained from *S. cerevisiae* TISTR 5027 grown in YM + peptone for 72 h were found to be significantly increased when the temperature was shifted from 30 °C to 37 °C ($p < 0.05$). According to this experiment, the highest yield of β-glucans of 40.96 ± 0.31 (% w/w) was achieved at 37 °C while the lowest of 37.50 ± 0.54 (% w/w) was achieved at 30 °C.

HaCaT is a keratinocyte located at the dermis. Generally, it is exposed to the environment and has a skin barrier as a defensive system protecting the skin from toxic environments or substances. SOD is a kind of antioxidant enzyme and acts as a defensive system for free radical production from the environment. NHDF is a fibroblast cell located at the dermis. Therefore, NHDF cells were used to evaluate collagen and elastin production.

The viability of HaCaT and NHDF cells after being exposed to various concentrations of extracted β-glucans is shown in Table 1. From the results, almost every treated concentration showed the percentages of cell viability higher than 70%, which is considered the passing criteria for the cytotoxicity test, except for the high concentration at 200.00 µg/ml of extracted β-glucan. This concentration is toxic to the cells because the percentage of cell viability was 66.93 ± 3.72%. Because NHDF cells were the primary unmodified cells, they showed greater sensitivity to the extracted β-glucan and less cell viability than those of HaCaT cells, which is an immortalized cell line [24]. Three concentrations above 25.00 µg/ml of extracted β-glucans showed cell viabilities of more than 70%, which are acceptable safety criteria according to ISO 10993-5 and safe for the used application. This suggested that the suitable treated concentrations of extracted β-glucans for HaCaT and NHDF cells were 100.00 and 25.00 µg/ml, respectively. In the next experiment, the efficiency of cellular components such as SOD, collagen, and elastin produced by activation with these concentrations of extracted β-glucans, would be determined.

From the results shown in Table 2, it is evident that the SOD activities of HaCaT cells activated by the extracted β-glucans at the concentration of 25.00, 50.00, and 100.00 µg/ml were significantly different from those of non-treatment cells ($p < 0.05$); however, only 100 µg/ml of the extracted β-glucan showed a positive result (31.82 ± 3.06 U/mg protein), compared to the non-treatment cells (27.57 ± 1.62 U/mg protein). For the extracted β-glucans at the concentration of 25.00 and 50.00 µg/ml, the SOD activities were slightly lower than those of non-treatment cells. The 10.00 µg/ml of standard SOD could activate the SOD activity at 72.30 ± 19.36 U/mg protein, while the activity of the concentration of 100.00 µg/ml was lower than that of the non-treatment. These results suggested that 100 µg/ml of the extracted β-glucan was the best concentration to activate the SOD activity of HaCaT cells, but it was not as good as standard SOD. Additionally, the SOD activities were related to the protein contents found in each treatment.

Results shown in Table 3 reveal that the amounts of cellular collagen and elastin produced by NHDF cells after challenging with 12.50 and 25.00 µg/ml of extracted β-glucans were higher than that of untreated cells. Unfortunately, this was not the case for the NHDF cells treated with 6.25 µg/ml of extracted β-glucans. The amounts of cellular collagen and elastin produced were the same as those found in the untreated cells. The difference in production was less than 0.1 µg/ml.

Table 1 Cell viabilities of HaCaT and NHDF after exposure to various concentrations of extracted β-glucans under 5% carbon dioxide at 37 °C for 72 h.

Treatment	% Cell viability of	
	HaCaT	NHDF
Non-treatment	100.00 ± 0.03^{b}	100.00 ± 0.05^{e}
β-Glucans (3.13 µg/ml)	105.18 ± 6.78^{b}	$92.68 \pm 3.75^{d,e}$
β-Glucans (6.25 µg/ml)	107.99 ± 7.31^{c}	85.25 ± 7.37^{d}
β-Glucans (12.50 µg/ml)	111.10 ± 7.29^{d}	68.33 ± 10.09^{c}
β-Glucans (25.00 µg/ml)	121.67 ± 6.02^{d}	73.71 ± 3.40^{c}
β-Glucans (50.00 µg/ml)	115.93 ± 2.85^{d}	56.54 ± 6.00^{b}
β-Glucans (100.00 µg/ml)	103.38 ± 6.33^{b}	44.97 ± 4.32^{a}
β-Glucans (200.00 µg/ml)	66.93 ± 3.72^{a}	41.07 ± 1.31^{a}

Results were expressed in mean ± SD, where $n = 3$. Significant differences for each treatment were determined by one-way ANOVA and Tukey's post-hoc tests, compared to non-treatment. a, b, c, d, and e indicate a significant difference ($p < 0.05$).

Table 2 SOD activity and protein content of HaCaT cells after elicited by extracted β-glucan at 37 °C for 30 min.

Treatment	SOD activity (U/mg protein)	Protein content (mg/ml)
Non-treatment	27.57 ± 1.62^{c}	0.846 ± 0.06^{c}
β-Glucan (25.00 µg/ml)	24.07 ± 1.98^{a}	0.781 ± 0.05^{b}
β-Glucan (50.00 µg/ml)	26.57 ± 0.17^{b}	0.821 ± 0.02^{c}
β-Glucan (100.00 µg/ml)	31.82 ± 3.06^{d}	0.758 ± 0.05^{b}
Standard SOD (10.00 µg/ml)	72.30 ± 19.36^{e}	0.388 ± 0.03^{a}

Results were expressed in mean ± SD, where $n = 3$. Significant differences for each treatment were determined by one-way ANOVA and Tukey's post-hoc tests, compared to non-treatment. a, b, c, d, and e indicate significant difference ($p < 0.05$).

Table 3 Amounts of cellular collagen and elastin produced by NHDF cells elicited from β-glucan extracted at 37 °C for 3 h.

Treatment	Cellular collagen produced by NHDF cells (μg/ml)	Elastin produced by NHDF cells (μg/ml)
Non-treatment	2.82 ± 0.13^{b}	78.71 ± 3.60^{b}
β-Glucan (6.25 μg/ml)	2.72 ± 0.10^{a}	78.32 ± 3.75^{a}
β-Glucan (12.50 μg/ml)	3.22 ± 0.08^{c}	86.12 ± 1.62^{d}
β-Glucan (25.00 μg/ml)	3.91 ± 0.17^{d}	84.11 ± 6.02^{c}
Ascorbic acid (25.00 μg/ml)	8.60 ± 0.11^{e}	119.74 ± 5.86^{e}

Results were expressed in mean ± SD, where $n = 3$. Significant differences for each treatment were determined by one-way ANOVA and Tukey's post-hoc tests compared to non-treatment. a, b, c, d, and e indicate significant difference ($p < 0.05$).

However, the amounts of cellular collagen and elastin activated by 12.50 and 25.00 μg/ml of β-glucans were lower than the result of the activation by 25.00 μg/ml of ascorbic acid.

DISCUSSION

Yeast cells frequently showed the highest amount of β-glucan at a stationary phase of cell growth; however, all yeast strains used in this study displayed different amounts of β-glucan in the range of 11.74 to 40.60 mg/100 mg of yeast cell dry weight within 7–9 h. This suggested that the amount of β-glucan depends on yeast strains rather than stage of cell, as reported by Jesus Torres-Osorno et al [5] that the compositions and amounts of β-glucan derived from 44 yeast isolates were diverse due to their species and geography.

To increase the β-glucan content, which is mainly located in the yeast cell wall [25], YM medium supplemented with 4 sources of essential elements for the biosynthetic of β-glucan were used for culturing *S. cerevisiae* TISTR 5027. Surprisingly, adding peptone and $(NH_4)_2SO_4$ to YM medium yielded better results in increasing yeast cell dry weight than adding glucose, which is the main component of the β-glucan backbone. This phenomenon was also observed by Aguilar-Uscanga et al [26], reporting that the amounts of yeast cell wall obtained from yeast cultured in various sources of medium were different. Yeast cultured in a medium containing only carbon sources such as glucose, mannose, sucrose, maltose, and galactose gained less cell wall than that cultured in media containing nitrogen sources e.g., peptone. Moreover, YM supplemented with yeast extract with high amino acid contents yielded less yeast cell dry weight than that with peptone and $(NH_4)_2SO_4$ which contain peptide and nitrogen sources; however, the reason for these phenomena is still unknown.

Aeration rates and temperatures are reported as the main physical parameters influencing β-glucan production by yeast [27]. Increasing the aeration rate during cell culture should result in a higher yield of β-glucan [28]. Unfortunately, the results obtained suggested that aeration rates did not significantly affect the amount of β-glucan obtained, as expected. This is different from the case of temperature. As the optimum temperature for growth of yeast cells is normally 25–30 °C [29], the maximum β-glucan was derived from the cells cultured at 37 °C instead of at 30 °C. The shift in temperature increased the production of β-glucan by nearly 40%. This might be attributable to the effects of temperature on cell wall integrity. Several studies suggested that high temperature is an adverse environment that affects the synthesis and assembly of the cell wall [30]. A rigid cell wall structure is required for cell survival. As β-glucan is the major component of the inner cell wall and can maintain cell wall integrity and morphology [31], increasing the β-glucan content might alleviate the stress induced by high temperature on yeast cell wall structure.

Oxidative stress is a major cause of various dermatological conditions, especially skin aging [32]. Use of β-glucan as a cosmetic ingredient helps defend against oxidative stress and regenerate collagen/elastin-producing cells [33]. Based on the SOD activity of β-glucan toward HaCaT cells, 100 μg/ml of β-glucan was required for the activation of SOD activity. It indicated that the amount of β-glucan, which was higher than the maximum safe concentration (> 100 μg/ml), needs to be used for reducing oxidative stress for skin aging. The less oxidative stress of the β-glucan might involve the structure of yeast β-glucan. Kofuji et al reported that β-glucan extracted from barley which mainly comprises β-(1,3-1,4)-D glucan showed stronger antioxidant effects than those from black yeast, which mainly consists of β-(1,3-1,6)-D glucan [34]. It appears that antioxidant activity was influenced by the structure of β-glucan. However, the mode of action of β-glucan *vis-a-vis* SOD activity is still unclear.

Concerning the production of collagen and elastin triggered by the extracted β-glucan, this mechanism seemed to have occurred through specific host receptors called pattern recognition receptors (PRRs) such as the toll-like receptor (TLR)-2/6, dectrin-1, or CR-3 on the surface of keratinocytes and fibroblast cells. As the structure of β-(1,3-1,6)-D glucan is that of a group of molecules known as pathogen-associated molecular patterns (PAMPs) [13], it can be bound to PRRs to activate the production of many cytokines. For example, Transforming Growth Factor- β (TGF-

β) is one of the most potent stimulators of collagen synthesis [35]. Although the β-glucan obtained in this study contains skin regenerative properties that involve a reduction of oxidative stress and activation of collagen/elastin-producing cells, the development of β-glucan as a cosmetic ingredient for reducing aging and wrinkles still needs some structural modification and future clinical studies to gain better results and more information.

CONCLUSION

The best yeast strain derived from the TISTR Culture Collection for β-glucan production in this study was *S. cerevisiae* TISTR 5027. The amount of β-glucan obtained during the stationary phase cell after a 7–9 h culture was 40.6064 mg/100 mg yeast cell dry weight. Adding peptone to the YM medium can increase the yeast cell dry weight by approximately 1.1-fold. About 4–6% of β-glucan content in this study can be enhanced by shifting the temperature from 30 °C to 37 °C. Importantly, β-glucan derived from this study can be used as a cosmetic ingredient for reducing aging and wrinkles because it can activate SOD activity and also promote the production of collagen and elastin from both studies; however, the chemical, physical, and biological modification of β-glucan to increase solubility or reduce molecular mass may require enhancing its activity in future studies.

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