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Effect of three additives on the cell morphology and β-glucan production in *Saccharomyces cerevisiae*

Naruemon Mongkontanawat[1], Romanee Sanguandeekul[1*], Cheunjit Prakitchaiwattana[1], Hang Xiao[2], Lynne A. McLandsborough[2] and Pawadee Methacanon[3]

[1] Dept. of Food Technology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Bangkok 10330, Thailand
[2] Dept. of Food Science, University of Massachusetts, Amherst 01003, USA
[3] National Metal and Materials Technology Center (MTEC), Thailand Science Park, Pathumthani 12120, Thailand.

ABSTRACT

The *S. cerevisiae* Angel® isolate was evaluated after culturing with the three different additives, each one and in combination, at three different concentrations, for a high growth rate and cell production. From this data, four optimal treatments were derived using MATHEMATICA computer program to study the effect of the type and concentration of additives on yeast cell morphology and β-glucan production. The lowest elongation (0.84 μm; rounder shape) and highest number of bud scars and β-glucan content (8.16% w/w 1.4-fold higher than the control) were found with 100 μg/ml SDS. Normal elongation (1.71 μm) and a 1.27-fold higher β-glucan content than the control was found when the yeast was cocultured with 20 μg/ml SDS and 3,000 μg/ml NaCl. No significant difference in cell elongation or correlation with the β-glucan yield was seen in all other treatments. The inclusion of 20 - 100 μg/ml SDS affected the cell shape, activated the budding process and led to an increase in β-glucan content. From these results, the addition of SDS in growth medium might be a simple method to enhance β-glucan production in yeast.

Keywords: Additives, Cell shape, β-glucan, *S. cerevisiae*, Budding process

[*] ***Corresponding author:***
E-mail: sromanee@chula.ac.th

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INTRODUCTION

β-Glucans are group of polysaccharides that are composed of glucose units linked together with beta-glycosidic bonds [1]. They have been used in many industries, such as the pharmaceutical, food and feed, and cosmetics industries [2-4]. β-Glucans also exhibit medicinal properties, such as antitumor, antimicrobial and antioxidant activities plus mycotoxin absorption [5-6], as well as uses in stimulation of the immune response in animals, such as shrimp, weaned pigs and mice, and the reduction of blood cholesterol and glucose levels [7-10]. The effect of immunoadjuvants has been reported to depend upon their structure, molecular weight and degree of branching, with long chain branching of β-glucans being the most effective [5,6].

β-Glucans have been found in many natural sources, such as bacteria, yeast, fungi and plants, but differ in their structure and functional properties [6]. The cell wall of *Saccharomyces cerevisiae*, budding yeast in the Saccharomycetaceae family, has been considered as an interesting source of β-glucan. It is approximately 30% of the cell dry weight and is comprised of 15% protein and 85% polysaccharide. β-Glucan has been shown to make 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[1].

S. cerevisiae can be easily and rapidly grown in a diverse array of culture media at a low production cost and its whole genome is already known. The β-glucan from *S. cerevisiae* has various properties that are more preferable to those found in other sources [11]. For example, the long branched side chain structure of β-glucan from *S. cerevisiae* has been shown to be the most effective immune enhancing source and it can improve the functional properties of some food products [3,4, 12-15]. 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, general, and no specific information on the effect of additives on β-glucan production in *S. cerevisiae* but rather the stimulation of β-glucan production in other organisms. For instance, *Botryosphaeria rhodina* was stimulated to produce β-glucan by the use of emulsified media [16], whilst induction of β-glucan synthetase in mushrooms was attained by culturing them in olive mill wastewater [17].

There are several factors that influence the morphology and components of the cell wall during the growth process, including the type of culture medium, carbon source, pH, temperature, aeration rate and culturing conditions. β-Glucan production by *S. cerevisiae* in a batch fermentor was found to be optimal when 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 [18]. Moreover, Congo red could directly inhibit β-glucan synthesis in the protoplast [19].

Studies on the enzymic and genetic mechanisms associated with β-glucan synthesis have reported that the addition of EDTA, fluoride and GTP to the culture medium stimulated β-

1,3-glucan synthase activity in *S. cerevisiae* [20], and that this was maximal with the addition of both EDTA and GTP [21]. Several reports state that NaCl could stimulate phosphoglucomutase (PGM2) and uridine diphosphoglucose pyrophosphorylase (UGPase) activity, which are enzymes involved in the UDP-glucose synthesis and trehalose accumulation. In addition increasing the saline level also increased ACT1 gene translation (actin biosynthesis) [22-23], but stimulated trehalose turnover [24]. Furthermore, 0.003 % w/v SDS, 3 mg/l hygromycin B and 3 μg/ml of the K1 killer toxin were reported to increase the β-1,6-glucan and total β-glucan levels when used as additives during *S. cerevisiae* growth on YPD agar plates[25]. Moreover, 0.02% w/v SDS stimulated FKS1 gene translation and actin cytoskeleton formation [26]. Thus, it is clear that additives that cause stress conditions can stimulate the yeast β-1, 3-glucan synthase activity *in vitro*. Consequently, β-glucan production may be stimulated through these mechanisms. Nevertheless, there is currently no information about the effect of additives on the β-glucan yield. Therefore, in this study, we investigated the effect of EDTA, SDS and NaCl, three additives known to enhance β-1,3-glucan synthase activity, on the correlation of the growth of *S. cerevisiae*, cell morphology and its β-glucan production.

MATERIALS AND METHOD

Yeast strains selection

Two commercial baker's yeasts, *S. cerevisiae* Fermipan® and *S. cerevisiae* Angel®, plus *S. cerevisiae* TISTR 5059 from the Microbiological resources center, Thailand Institute of Scientific and Technological Research, were used in this study.

Yeasts were cultured in YPD medium (Himedia, India), adjusted to pH 4.0, at 30 °C for 48 h with shaking at 200 rpm [18]. Yeast cells were collected by centrifugation at 8000 x g for 10 min, the yeast pellet harvested and freeze-dried prior to being subjected to β-glucan content analysis using a Yeast Beta-Glucan Assay Kit (Megazyme, Ireland) and determining the % yield of yeast cell dry weight [27,28].

Effect of the additive concentration on yeast growth

The yeast strain selected from the previous section was cultured in YPD medium, as described above, with the supplement of additives EDTA (0, 50 and 100 μg/ml; Fisher Scientific), SDS (0, 100 and 200 μg/ml; Fluka Biochemica) and NaCl (0, 30,000 and 60,000 μg/ml; Fisher Scientific). Growth was determined by serial dilutions of the cultures on to YPD plates every 8 h for 48 h [29], and after culturing at 30^{o}C for 24 - 48 h, counting the number of colonies on each plate. The yeast growth rate and corresponding growth model were then estimated by using non-linear regression. Sigmoid curves of growth can be explained with a shifted logistic equation [30-32], as shown in eq. 1 below:

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$$\Delta y_{(t)} = \Delta y_{asym}\left\{\frac{1}{1+\exp[k(t_c - t)]} - \frac{1}{1+\exp[kt_c]}\right\} \quad (1)$$

where $\Delta y_{(t)}$ represents the adjusted ratio of log $N_{(t)}/N_O$ at time (t), Δy_{asym} represents the adjusted ratio of log $N_{(t)}/N_O$, which roughly represents the growth level at the stationary phase, t_c represents the time to reach the highest growth rate, that is the sigmoidal curve's inflection point's location, and k is its slope at this point, representing the growth rate. Optimum concentrations of different combinations of the additives were then further estimated using the MATHEMATICA computer program [33].

Determination of cell shape, wall surface and β-glucan production of yeast grown with additives

Yeast was cultured in YPD medium, adjusted to pH 4.0, and supplemented with the optimum concentrations of the three additives (EDTA, SDS and NaCl), as determined from the previous section, at 30 ^{o}C with shaking at 200 rpm for 24 h. Yeast cells were then sampled from the culture and photographed with a confocal microscope in differential interference contrast (DIC) mode at 400 X magnification (Nikon C1 Digital Eclipse, Japan). The images were analysed with ImageJ software (NIH Image, USA.) to determine the average size, as major and minor axes lengths, and elongation (calculated from the difference in the major and minor axis length) of every single cells, and thus the changes in cell shape [34].

Scanning electron microscopy (SEM) (JEOL, model JSM-5410LV, Japan) at a magnification of 5,000 X and 20,000 X was used to capture the changes in whole cell shape and cell wall surface, respectively, of yeasts grown under each culture condition. Evaluation of the β-glucan content was performed as described above.

Statistical analysis

All experiments were repeated in triplicate. The analyses of statistical significance between means or treatments was performed using the SPSS program version 17 (SPSS, Inc., Chicago, IL), where $p \leq 0.05$ was considered as statistically significant. The data were analysed with Analysis of Variance (ANOVA) and multiple comparisons with Duncan's Multiple range test [35].

RESULTS AND DISCUSSION

Yeast strains selection

The β-glucan production level of three different *S. cerevisiae* strains when grown under standard conditions (YPD media at pH 4.0 at 30 ^{o}C with shaking and aeration) are presented in Table 1. The *S. cerevisiae* Fermipan® isolate exhibited the highest cell dry weight, although this was not statistically different from that obtained with the *S. cerevisiae* Angel® isolate. On the

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other hand, the cell dry weight, obtained for the *S. cerevisiae* TISTR 5059 isolate was significantly lower. The β-glucan content from the three yeast isolates varied numerically, being highest in the *S. cerevisiae* Angel® isolate and lowest in the *S. cerevisiae* Fermipan®, but these were not statistically significantly different. The *S. cerevisiae* Angel® isolate was selected for evaluation of the effect of the three additives upon β-glucan production, since it could grow well and produced the highest amount of β-glucan relative to the other two strains.

Table 1. β-Glucan content (as % (w/w dry weight) of the cell) and the % yield of cell dry weight of *S. cerevisiae* Fermipan®, *S. cerevisiae* Angel® and *S. cerevisiae* TISTR 5059 isolates[*] when cultured for 48 h.

Strains	β-Glucan content (% w/w of cell)[**]	Yield (% of cell dry weight)[**]
***S. cerevisiae* Fermipan®**	7.10 ± 1.55[a]	0.43 ± 0.13[a]
***S. cerevisiae* Angel®**	8.95 ± 0.66[a]	0.35 ± 0.05[a]
***S. cerevisiae* TISTR 5059**	8.54 ± 1.15[a]	0.14 ± 0.03[b]

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

Effect of the additive concentrations on yeast growth

The *S. cerevisiae* Angel® isolate was cultured with three additives (EDTA, SDS and NaCl) at various concentrations and the parameters of the growth model are described in Table 2.

Table 2. The culture conditions and the parameters of the growth model from the *S. cerevisiae* Angel® isolate when cultured with EDTA, SDS and NaCl at different concentrations for 48 h

Treatments	Additives (μg/ml)			Growth[#]	Growth model[*,**]			
	EDTA	SDS	NaCl		Δy_{asym}	k CFU/h)	t_c (h)	R^2
1 (control)	0	0	0	G	3.63	0.24	10.87	1.00
2	0	100	0	G	3.67	0.22	12.13	1.00
3	0	200	0	G	3.77	0.25	12.73	0.95
4	50	0	0	G	3.50	0.24	12.16	1.00
5	50	100	0	D	__	__	__	__
6	50	200	0	D	__	__	__	__
7	100	0	0	G	3.24	0.21	10.30	0.99
8	100	100	0	D	__	__	__	__
9	100	200	0	D	__	__	__	__
10	0	0	30,000	G	2.73	0.36	17.38	1.00
11	0	100	30,000	D	__	__	__	__
12	0	200	30,000	D	__	__	__	__
13	50	0	30,000	G	3.11	0.07	24.41	1.00
14	50	100	30,000	NG	__	__	__	__
15	50	200	30,000	NG	__	__	__	__
16	100	0	30,000	NG	__	__	__	__
17	100	100	30,000	NG	__	__	__	__
18	100	200	30,000	NG	__	__	__	__

19	0	0	60,000	G	1.45	0.12	11.68	1.00
20	0	100	60,000	NG	__	__	__	__
21	0	200	60,000	NG	__	__	__	__
22	50	0	60,000	NG	__	__	__	__
23	50	100	60,000	NG	__	__	__	__
24	50	200	60,000	NG	__	__	__	__
25	100	0	60,000	G	0.86	0.23	7.82	0.99
26	100	100	60,000	NG	__	__	__	__
27	100	200	60,000	NG	__	__	__	__

*- could not be determined by using Shifted logistic equation, Δy_{asym} is dimensionless; k is in CFU per hour, and t_c in hours. **Data are shown as the mean and are derived from three replicates. #G is growth, NG is no growth, D is cell death,

A shifted logistic equation was used to explain the obtained sigmoid growth curve of the yeast, and from this the growth rate, growth level at the stationary phase and time to reach the highest growth rate are then explained with the k value, ? Δy_{asym} and t_c values, respectively. As shown in Table 2, the addition of SDS at 100 and 200 μg/ml (Treatments 2 & 3) gave a higher growth level with increasing SDS concentrations, represented by the higher ? Δy_{asym} value, when compared to the control even though the time to reach the highest growth rate was longer than the control and increased further for the higher SDS concentration. Combined with the low t_c and high k values, this indicates that the *S. cerevisiae* Angel ® isolate grew faster in the presence of SDS than in the presence of the other additives or the no-additive control. On the other hand, the addition of 50 and 100 μg/ml EDTA (Treatments 4 & 7) resulted in a reduction in the growth level (lower ? Δy_{asym} level) with increasing EDTA concentrations, combined with a similar k and higher t_c value than the control yeast culture. The yeast growth level was reduced to an even higher amount with the addition of 3 % w/v and, especially, 6 % w/v NaCl (Treatments 10 & 19) with a very low ? Δy_{asym} value. A much longer t_c and a slightly higher k value than the control. Overall, this indicates that this yeast isolate could still grow well in the presence of 50 μg/ml EDTA, 3 % w/v NaCl and, especially, 100 μg/ml SDS.

The addition of a combination of two or three of the additives was found to inhibit the growth of yeast to such a level that the ? Δy_{asym} value could not be calculated (Table 1). The two exceptions were 50 μg/ml EDTA, 3 % w/v NaCl (Treatment 13), and 100 μg/ml EDTA, 6 % w/v NaCl (Treatment 25). When the k value was evaluated, the growth rate obtained with the addition of 200 μg/ml SDS (Treatment 3) or 3 % w/v NaCl (Treatment 10) were slightly and significantly increased, respectively, when compared to the control. Apart from the culture with the addition of 50 μg/ml EDTA (Treatment 4) which showed the same growth rate as the control, the growth rate in the rest treatments were lower than the control. In terms of t_c, *S. cerevisiae* Angel® grew slower than the control when supplemented with 100 or 200 μg/ml SDS (Treatments 2 & 3), 50 μg/ml EDTA (Treatment 4), 3 or 6 % w/v NaCl (Treatments 10 & 19), and the combination of 50 ppm EDTA and 3 % w/v NaCl (Treatment 13). Because the cells adapted to the new environmental conditions involves significant energy expenditure and / or actin depolymerization mechanisms might occur and so consequently the cell cycle was delayed [36-37]. The lag phase has been reported to be extended when *S. cerevisiae* is cultured with NaCl in the range of 14,610 - 87,660 μg/ml, as the yeast has to use more energy for maintaining the

electrochemical gradient of sodium ions, resulting in less energy being available for the anabolic metabolism required for cell division and so a lower culture growth rate was seen compared with the control [38]. However, in this study the yeast in the presence of 100 and 200 μg/ml SDS showed a higher growth level than that of the control. This may well be due to the influence of SDS, which at 0.02 % w/v (200 ppm) has been reported to increase the activity of the □-glucan synthase gene, FKS1, and the PIM1 gene that controls the actin cytoskeleton, and so increases the budding process in *S. cerevisiae* [26, 39]. In this scenario, cell wall components would be increasingly synthesized, leading to the increase in growth rate and growth level at stationary phase [26]. In the remaining cases of no cell growth and cell death, the growth of the yeast culture could not explained by using equation (1). From this case, it can be explained that high concentrations of the anionic detergent (SDS), divalent metal ion chelating agent (EDTA) and the hyperosmotic pressure (NaCl) affected the diffusion of the cell membrane and disturbed the medium osmolarity so as to irreversibly damage or impair the function of the cell wall [40]. Moreover, the chelation of divalent metal ions, such as Mg^{2+} and Ca^{2+}, by EDTA is problematic inside the cell where divalent metal ion dependent enzyme and signaling pathway activities can be impaired or blocked and consequently the yeast growth reduced [41]. In addition, in hyperosmotic conditions, water diffuses out of the cell, causing a change in the cell wall properties and cell shrinkage, with decreased cell viability [42].

Previous research, used as the basis of this work, had established that the toxic effects on the *S. cerevisiae* growth rate were found at concentrations of or above 400 μg/ml EDTA [41], 100 μg/ml SDS [43] and 85,000 μg/ml (8.5 % w/v) NaCl [44]. However, here the Angel ® yeast isolate could only tolerate these additives at a lower concentration, namely 100 μg/ml EDTA, 200 μg/ml SDS and 60,000 μg/ml (6 % w/v) NaCl. This could be due to the differences in tolerance between different yeast strains or the culture conditions used. In contrast, the cultures supplemented with 100 μg/ml EDTA (Treatment 7) and a combination of 100 μg/ml EDTA and 6 % w/v NaCl (Treatment 25) had a slightly and significantly lower t_c value than the control, respectively.

Based on the above parameters of the growth model for each condition, the optimum concentrations of single additives were set as 50 μg/ml EDTA, 100 μg/ml SDS and 3 % w/v NaCl. Therefore, the data from the growth rate (Table 2) were used to further calculate the predicted optimum conditions using the MATHEMATICA computer program. Consequently, the four additional conditions of a combination of (i) 5 μg/ml EDTA and 10 μg/ml SDS, (ii) 5 μg/ml EDTA and 3,000 μg/ml NaCl, (iii) 20 μg/ml of SDS and 3,000 μg/ml NaCl and (iv) 5 μg/ml EDTA, 10 μg/ml SDS and 3,000 μg/ml NaCl, were obtained. These were included in the eight culture growth conditions to be evaluated for the effect of additives on the cell shape and β-glucan production in the *S. cerevisiae* Angel® isolate (control and seven additive treatments), as shown in Table 3.

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Determination of cell shape, wall surface and β-glucan production of yeast growth supplemented with additives

The yeast cell shape, as determined by confocal microscopy (data not shown) and SEM at 400 X and 5,000 X magnification, respectively, (Figure 1), and the wall surfaces, as determined by SEM at 20,000 X magnification (Figure 2), were evaluated in the yeast growed in the control condition plus seven different stress inducing conditions as outlined in Table 3.

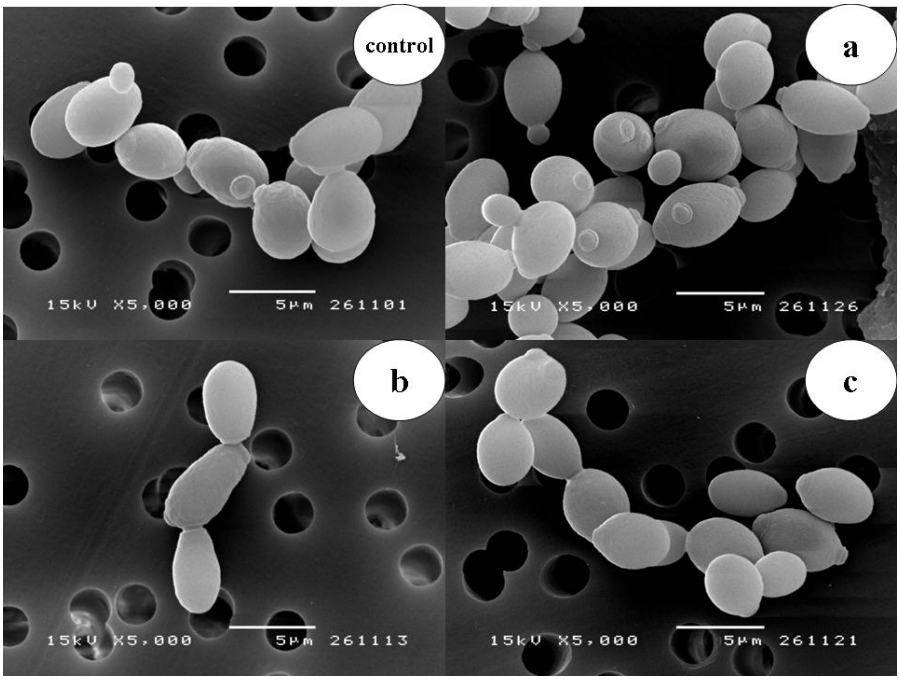

Figure 1. Representative SEM micrographs (5,000 x magnification) of the *S. cerevisiae* Angel® isolate when cultured in YPD media (control) or that supplemented with (a) 100 µg/ml SDS, (b) 5 µg/ml EDTA and 3,000 µg/ml NaCl and (c) 5 µg/ml EDTA, 10 µg/ml SDS and 3,000 µg/ml NaCl.

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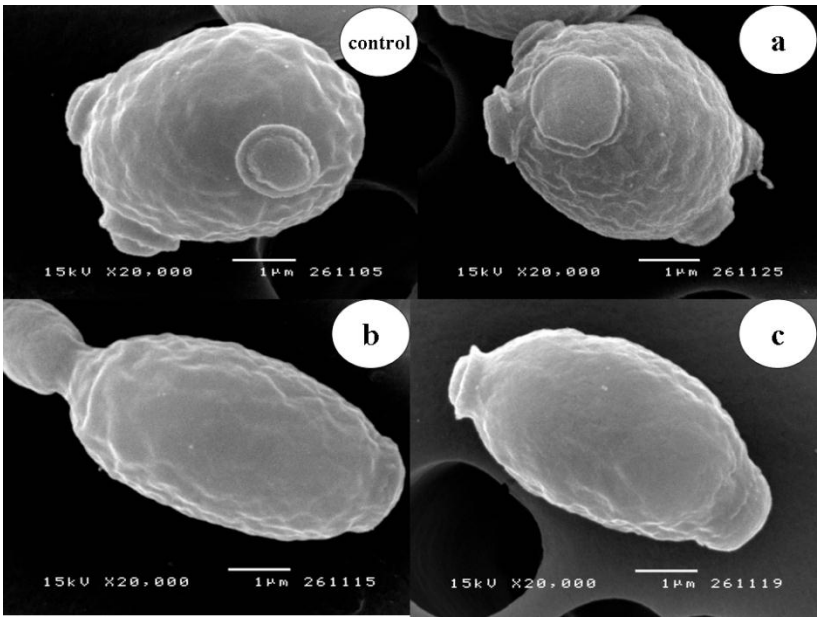

Figure 2. Representative SEM micrographs (20,000 x magnification) of the *S. cerevisiae* Angel® isolate when cultured in YPD media (control) or that supplemented with (a) 100 µg/ml SDS, (b) 5 µg/ml EDTA and 3,000 µg/ml NaCl and (c) 5 µg/ml EDTA, 10 µg/ml SDS and 3,000 µg/ml NaCl.

Based on the length of the two axes, the cell shape could be divided into three morphological groups. The first morphological group had an almost spherical shape with the major axis length, being less than 0.84 µm, longer than the minor axis length. These almost spherical cells were seen only when the yeast was cultured in the media supplemented with 100 µg/ml SDS (Treatment 2), and this almost rounder elongation pattern of single yeast cells was significantly different from that seen in all other treatments (Table 3 and Figures 1a). Base on these results, the destabilization by SDS of the cell wall may lead to the protective formation of a rigid rounder cell [45].

Table 3. Elongation of single cell and the β-glucan content of the *S. cerevisiae* Angel® isolate cultured in YPD medium supplemented with the indicated additives and concentrations for 24 h.[*]

Treatment	EDTA (µg/ml)	SDS (µg/ml)	NaCl (µg/ml)	Elongation of single cell (µm)[#]	Shape	Average single cell size (µm^2)	β-Glucan content (% w/w)[#]
1 (control)	0	0	0	1.79 ± 0.19^{ab}	oval	0.034	5.82 ± 0.30^{c}
2	0	100	0	0.84 ± 0.18^{c}	rounder	0.034	8.15 ± 0.87^{a}
3	50	0	0	1.51 ± 0.15^{b}	oval	0.034	6.33 ± 0.92^{bc}
4	0	0	30,000	1.72 ± 0.03^{ab}	oval	0.034	6.22 ± 0.16^{bc}
5	5	10	0	1.72 ± 0.12^{ab}	oval	0.034	6.56 ± 0.57^{bc}
6	5	0	3,000	2.00 ± 0.14^{a}	long oval	0.034	6.41 ± 0.71^{bc}
7	0	20	3,000	1.71 ± 0.23^{ab}	oval	0.034	7.39 ± 0.65^{ab}
8	5	10	3,000	1.61 ± 0.30^{b}	oval	0.034	6.69 ± 0.51^{bc}

[*]Data are shown as the mean ± one SD and are derived from three replicates. [#]Means within a column followed by a different letter are significantly different (P<0.05).

The second morphological group displayed a long oval shape and was found only in yeast cells cultured with the combined addition of 5 μg/ml EDTA and 3,000 μg/ml NaCl (Treatment 6), and revealed the longest elongation of the single cell of 2.0 ?m (Table 3 and Figures 1b & 2b). The third morphological group was in-between these two groups, the cells were oval shape and the control was also in this group (yeast cultured under standard low stress conditions) plus all the other five additions (Table 3 and Figures 1c & 2c). Nevertheless, no significant difference in the average single cell size was found among all treatments in the last two groups (Table 3).

When representative cells of each of the three morphological cell shapes (Treatments 2, 6 and other in Table 3 for morphology groups 1 (rounder), 2 (long oval) and 3 (oval), respectively), plus the control (no additives), were subjected to SEM analysis at 5,000 X magnification, the observed cell shapes were corresponded to that observed by confocal microscopy. Thus, the preparative procedure required for SEM did not cause any gross distortion in the yeast cell morphology. When the cell surface of these three representative yeast cultures plus the no-additive control culture were analysed by SEM at 20,000 X magnification, the results were somewhat different. Yeast cells grown in the presence of 100 μg/ml SDS with the noticeably rounder shaped morphology showed multiple bud scars on the wall surface, which were not found on the wall surfaces of the oval or long oval shape yeast cells (Table 3 and Figures 1a & 2a).

The β-glucan content from yeast grown in YPD supplemented with all seven treatments (Table 3) were higher than the control, especially with 100 μg/ml SDS. Perhaps under these conditions the yeasts increase the synthesis of cell wall components as remedial protection from the stress caused by the respective additive(s). The average β-glucan contents of the yeast cells was also divided into three clear groups which corresponded to the three cell morphology groups (Table 3). The β-glucan content of the almost rounder shaped yeast cells, found only in yeast grown in the presence of 100 μg/ml SDS, was significantly (1.4-fold) higher than the control, and around ~1.2-fold higher than that of the other groups. The average β-glucan content of the oval shaped yeast cells was numerically higher than that of the control and the long oval shaped cells but was not statistically significant except for those cells cultured with both 20 μg/ml SDS and 3,000 μg/ml NaCl. In the longest cell shape, the average cellular β-glucan content was also higher than that in the control yeast cells, but was not statistically different from that found in the oval shaped group. From this result, it can be explained that, β-glucan synthase is redistributed in response to the cell wall stress to repair general cell wall damage [1, 26, 36]. As previously described, 0.02% w/v (200 μg/ml) of SDS can stimulate depolarizing of the β-glucan synthase gene (FKS1) and activate PIM1, which is involved in MAPKs (Mitogen activated protein kinase pathway) in the yeast and so alter the actin cytoskeleton via activating actin patch and cables [26, 39, 46]. Consequently, an increase in the budding process, cell wall biosynthesis, the number of cells and the β-glucan content were then induced, as found in this study. In the case of 50 μg/ml EDTA, the resultant β-glucan content was slightly higher than that of the no-additive control cells, in accord with the reports of

another researchers, who found that the addition of EDTA to yeast cultures stimulated β-1,3-glucan synthase activity [20-21].

In the case of NaCl, it seemed that this slightly increase in β-glucan levels (7% higher than the control level), could activate enzymes associated with UDP-glucose synthesis, resulting in increased β-glucan synthesis. Moreover, NaCl also stimulated ACT1, one of the actin production control genes and increased in production of cell wall thickness in *S. cerevisiae* under hyperosmotic conditions [22,23, 42].

CONCLUSION

From our results, we propose that the addition of 100 ppm SDS to the YPD culture medium, stimulated *S. cerevisiae* growth by accelerating the budding process. During the budding, the cell wall components, including β-glucan, were likely to be rapidly synthesized leading to a rounder cell shape and rapid budding process. Interestingly, the oval or long oval shaped cells observed in the conditions of 50 μg/ml EDTA, combination of 5 ppm EDTA and 3,000 μg/ml NaCl and were predicted to have grown slower than the SDS condition, generating a significantly lower β-glucan content than the rounder shaped cells. The β-glucan content among the cells of a similar morphology from these different culture conditions were not significantly different, supporting that the β-glucan content is correlated to the cell shape as a result of the budding rate, which is itself stimulated by the additives. This observation requires further confirmation but is relatively novel being also the first report about the correlation among cell growth, cell morphology and β-glucan production in *S. cerevisiae*. Overall, this could form the basis for developing more efficient methods to apply in the yeast β-glucan production for industry. However, the systematic investigation about the effect of the additives on β-glucan quality, and in particular its functional properties, is required to confirm the efficiency of using SDS for improving *S. cerevisiae* β-glucan production.

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