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Enhancement of Antioxidant Glutathione Production by *Saccharomyces cerevisiae*scc Growing under Stressful Condition

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Abstract: The cultivation of *Saccharomyces cerevisiae*(SCC) in a medium containing molasses as carbon source gave low content of glutathione (GSH) (antioxidant compound). The highest antioxidant level was observed on basal medium containing ethanol as a sole carbon source followed by medium containing glycerol being 423 and 335μ mol/g dry cell, resulting to 17.62and 13.95 μ mol/g/h for antioxidant productivity. The sequence of total antioxidant capacity values were: ethanol > glycerol > glucose > sucrose > molasses. The cultivation of *S. cerevisiae* SCC in a medium containing 0.1 or 0.5 mg/ml furfural gave the highest values of antioxidant capacity being 345 and 335 µ mole/g, respectively. Examined yeast isolate was grown on media containing different concentrations of sodium chloride. At 1 and 2 % NaCl, cell dry weight of SCC was slightly affected when compared with control. The maximum content of glutathione (18 mg/g dry cells) was recorded at 1% NaCl. On the other hand, glycerol and gassing power were increased by increasing NaCl concentration in a growth medium and reached the maximum of 220 mg/g dry cell and 44 cm^3 at 2 % NaCl, respectively. In the same trend, the increasing of osmotic stress in the medium increased glutathione content, glycerol and the gassing power. The highest values of GSH content was recorded after 120 min in a medium containing 10 % glucose followed by 20 % being 35.32 and 26.0 mg/g dry cell, respectively. The maximum glycerol content was recorded at 90min in a medium containing 30 % glucose being 230 mg/g dry cell. The high values of gassing power were recorded to be 66 cm^3 after 30 min and 71 cm^3 after 60 min incubation. This condition improved the GSH antioxidant, glycerol content and gassing power of the studied yeast isolate.

Key words: Saccharomycescerevisiae (SCC) · Antioxidants · Glutathionegsh · Gassing Power

INTRODUCTION

The formation of several antioxidants can be induced by *Saccharomyces cerevisiae* grown under stressful conditions such as high NaCl concentration, medium high osmotic potential or in response to fermentation medium ingredients such as some carbon sources, phenolic compounds or by additions of substance that are known to be toxic to cells growth aerobically [1-3].

Recently, the increased interest in natural antioxidants has given rise to the screening of microbial sources to replace the synthetic compounds currently in use as food antioxidants. Natural antioxidants can also be used in nutraceutical applications as supplements. Natural anti-oxidants are presumed to be safer for human beings. Yeasts synthesize a number of bioactive compounds which can serve as antioxidants. These have found numerous uses in foods to retard oxidative degeneration of fatty substances and in nutraceutical supplements to improve health and well-being. They consist of the oxygenated carotenoid torulahodin, both the organic acid and the salt forms of citric acid, coenzyme Q or ubiquinone, glutathione, hydroxymethyl and hydroxylethylfuranone (2H), tocotrienol, a-tocopherols (a-TOHs) and other forms of tocopherols, riboflavin (vitamin B2) and the flavins derived from it and 2,4-hydroxyphenyl ethanol [4].

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Recent data also suggest that polysaccharides reveal antioxidant activity that can result in their protective function as antioxidants, anti mutagens and antigenotoxic agents. The derivatives of beta-D-glucan demonstrated potent inhibitory effect on lipid peroxidation comparable to that of the known antioxidants and exerted DNA protection from oxidative damage. The results indicate significant protective antioxidant, antimutagenic and antigenotoxic activities of the yeast polysaccharides and imply their potential application in anticancer prevention/therapy [5].

Glutathione (GSH) has multi usage, i.e., as a protein flavoring, antibiotic and antioxidant and its use as coenzyme and enzyme in various types of biochemical reactions such as oxidization, reduction and antitoxin. GSH can also be used as an antitoxin of oxidized substances that are produced by the oxidization process of selenium inside human body which can cause cancer. In humans, GSH deficiency can be associated with many disease states, such as liver cirrhosis, pulmonary diseases, gastrointestinal and pancreatic inflammations, diabetes, neurodegenerative diseases and aging. Nowadays, GSH is widely used as a drug and has great potential to be used in food additives and in the cosmetic industries [6].

GSH is still not being commercially used for its high price which can be reduced further as many studies had been done to increase the yield of GSH production. Glutathione is the most abundant non-protein thiol compound present in living organisms. This compound can be produced using enzymatic methods in the presence of ATP and its three precursor amino acids (L-glutamic acid, L-cysteine, glycine) [7].

Therefore, the aim of this work is to study the effect of different carbon sources, furfural, NaCl and a medium osmotic stress on the level of total antioxidant capacity in the tested cells *S.cerevisiae* (SCS).

MATERIALS AND METHODS

Source of Baker's Yeast Isolates: Yeast isolates were isolated from commercial Baker's yeast packets using streaking plate method on Yeast Extract Peptone Dextrose (YPD) agar [8] grown at 30°C for 24-48 h. The developed yeast colonies were then picked up and examined microscopically in wet preparation and by gram staining to check their purity. The cultures were maintained at 4°C and sub cultured monthly on YPD or basal medium agar slants.

Effect of Carbon Source: This experiment was designed to study the effect of different carbon sources on the total antioxidant capacity of yeast cells. Therefore, trials were done to replace the glucose in basal medium by four carbon sources individually in amount equal to that present in the original medium. Carbon source applied was sucrose, glycerol, molasses or ethanol. At the end of fermentation time (24 h)on a rotary shaker (Lab line shaker incubator)at 150 rpm/min using shake flask as a batch culture, the cell dry weight and total antioxidant capacity were determined according to Li, Wei and Chen [7].

Effect of Furfural: The effect of different concentrations of furfural ranged from 0.1 to 1.0 mg /ml medium on the total antioxidant capacity of yeast cells were tested according to Peng *et al.* [9].

Effect of Sodium Chloride (NaCl): Different concentrations of sodium chloride ranging from 1 to 5 % were used to study the effect of salt stress on the growth of *S.cereviciae* SCS strain in basal medium containing molasses as a carbon source. Themolasses was used as the molasses solution according to Roukas [10]. After 24 h, cells were harvested by centrifugation at 5000 rpm for 10 min and then washed twice with tap water and centrifuged again. The cell dry weight, gassing power, glycerol content and reduced glutathione concentration were determined.

Effect of Hyperosmotic Stress: In this experiment, yeast cells produced from fermenter as a batch culture were exposed to hyperosmotic stress to study their effects on the antioxidant substance (Reduced glutathione) of yeast cells. The yeast cells were suspended in hyperosmotic medium containing 10, 20 and 30 % glucose to give an optical density 10 at 620 nm and kept at 25°C. Samples were taken after different times and centrifuged at 5000 rpm for 10 min to determine the cell dry weight, gassing power, glutathione (GSH) and glycerol content in the cells as described by Reed *et al.* [11].

Bioreactor as a Batch Culture: The final working volume was 2 L, temperature; aeration, pH and speed of agitation were set on 30°C, 80 % saturation O_2, 4.5 and 500 rpm respectively. During fermentation, samples (10-20 ml) were periodically withdrawn from the culture (fermentation vessel). The cells dry weight was determined as previously mentioned.

RESULTS AND DISCUSSION

Effect Carbon Source: Data presented in Table 1. clearly showed that the highest antioxidant level was observed on basal medium containing ethanol as a sole carbon source followed by a medium containing glycerol being 423 and 335μ mole/g dry weight, resulting to 17.62 and 13.95 μ mol/g/h for antioxidant productivity. Also, the medium which gave the highest cell dry weight (containing molasses, 6.4 g/l, or sucrose, 7.0 g/l) did not improve the formation of antioxidant in the cell and led to decrease the antioxidant productivity by 6.75 and 6.04 % respectively, as compared with medium containing ethanol as a carbon source. The sequence of total antioxidant capacity values were: ethanol > glycerol > glucose > sucrose > molasses.

This finding may be attributed to fermentable carbon source, especially glucose, deriving its energy from glycolysis and making negligible use of its mitochondria, or on non fermentable substrates like glycerol and ethanol, activating its mitochondrial oxidative metabolism. In the latter case, the mitochondrial respiratory chain is in operation and the production of reactive oxygen species can be expected to be significantly augmented as an unavoidable by-product of oxidation, assuming the mitochondria are the main cellular sources of superoxide. The control of yeast metabolic pathways is complex, involving not only the induction/repression of enzymes directly active in the metabolism of the appropriate substrates, but also affecting the level of antioxidants and antioxidant enzymes [12].

Effect of Furfural: The commercial production of baker's yeast all over the world is done by using heated molasses as a main substance for growth. The heating process resulting to formation furfural, so, the effect of different concentration of furfural on the level of antioxidant in yeast cells was the goal of this experiment. Results in Table 2 clearly showed that, the cultivation *S.cerevisiae* SCC in a medium containing furfural 0.1 or 0.5 mg/ml medium gave the highest values of antioxidant capacity being 345 and 335 μ mol/g, respectively. The corresponding values of productivity were 14.37 and 13.95 μ mol/g/h, respectively. Also, the cultivation of *S.cerevisiae* SCC on furfural (0.1 and 0.5 mg/ml) improved the antioxidant capacity 15 and 11.66 % as compared with control. Increasing concentration of furfural higher than 0.5 mg/ml led to decrease the

Table 1: Effects of different carbon sources on the growth of *S.cerevisiae* SCC and total antioxidant capacity after 24 h incubationat 30°Cusing shake flasks as a batch culture

Carbon source	Biomass (g/l)	Total antioxidant capacity (μ mol/ g cell dry weight)	Antioxidant Productivity (μ mol/g/h)
Ethanol	2.54 ± 0.2	423 ± 23	17.62 ± 0.95
Glycerol	3.45 ± 0.4	335 ± 22	13.95 ± 0.91
Glucose	5.0 ± 0.4	300 ± 18	12.50 ± 0.75
Molasses	6.4 ± 0.3	166 ± 12	6.91 ± 0.50
Sucrose	7.0 ± 0.2	169 ± 13	7.04 ± 0.54

Table 2: Effects offurfural concentration on level and productivity of total antioxidant capacity after 24 h incubation at 30°Cusing shake flask as a batch culture

Concentration of furfural (mg/ml)	Total antioxidant capacity (μ mol/ gcell dry weight)	Antioxidant Productivity (μ mol/g/h)
0(control)	300 ± 17	12.50 ± 0.71
0.1	345 ± 15	14.37 ± 0.62
0.5	335 ± 11	13.95 ± 0.46
1.0	160 ± 9.0	6.66 ± 0.37

Table 3: Effect of NaCl concentration on biomass of *Saccharomyces* cereviciae, content of GSH and glycerol growing on molasses medium in(batch culture) at 30°C

Concentration of NaCl (%)	Biomass (g/l)	Content of GSH (mg/g cell dry weight)	Content of glycerol (mg/g cell dry weight)	Gassing power (Cm^3)
0	5.0 ± 0.41	13 ± 1.1	25.0 ± 1.5	39 ± 2.0
1	4.10± 0.32	18 ± 0.9	86.4 ± 2.1	41± 2.0
2	2.70 ± 0.25	17 ±1.0	220 ± 2.4	44 ± 2.0
3	1.03 ± 0.16	7.7 ± 0.87	207 ± 2.7	32 ± 1.5
4	0.64 ± 0.08	6.0 ±1.0	109 ± 2.9	20 ± 2.2
5	0.040	0	0	0

Table 4: Effect of hyperosmotic stress on content of glutathione in *S.cerevisiae* SCC cultured on molasses medium in a fermenter 30°C as batch culture

	Glutathione (mg/g cell dry weight)content at different glucose concentrations			
Incubation time (min)	0 % glucose	10 % glucose	20 % glucose	30 % glucose
0	14.50 ± 0.27	14.80 ± 0.61	14.50 ± 0.7	14.30 ± 0.60
30	14.30 ± 0.25	15.80 ± 0.63	11.66 ± 0.6	10.33 ± 0.60
60	14.60 ± 0.45	20.50 ± 0.55	14.50 ± 0.3	12.16 ± 0.4
90	14.50 ± 0.29	26.83 ± 0.85	22.0 ± 0.6	15.50 ± 0.55
120	14.70 ± 0.43	35.32 ± 0.65	26.0 ± 0.82	18.63 ± 0.55
150	14.60 ± 0.28	35.30 ± 0.72	25.60 ± 0.65	21.50 ± 0.52
180	14.50 ± 0.41	34.16 ± 0.91	24.16 ± 0.73	24.30 ± 0.70
210	14.60 ± 0.25	31.16 ± 0.95	21.16 ± 0.63	24.20 ± 0.20

Table 5: Effect of hyperosmotic potential on content of glycerol in *S.cerevisiae* SCC cultured on molasses medium in a fermenter at 30°C as batch culture

	Glycerol content(mg/g cell dry weight) at different glucose concentrations			
Incubation time (min)	0 % glucose	10 % glucose	20 % glucose	30 % glucose
0	18 ±1.58	18.4 ±1.5	18 ±1.8	18 ± 2.2
30	18 ±1.87	202 ± 4.5	220 ± 4.4	206 ± 2.0
60	20 ±1.74	200 ± 5.0	222 ± 5.1	228 ± 5.0
90	17 ±1.58	182 ± 6.0	198 ± 4.1	230 ± 3.5
120	16 ±1.85	172 ± 4.8	180 ± 3.8	200 ± 3.0

antioxidant capacity being 53.62 % as compared with medium containing 0.1 mg/ml furfural or 52.23 % as compared with medium containing 0.5 mg/ml furfural, respectively.

These results are in agreement with previous observations concerning the effect of furfural on yeast byLiu *et al.* [13] and Kelly *et al.* [14].

Effect of Sodium Chloride: Yeast cells were cultured on molasses medium supplemented with different concentrations of sodium chloride (1, 2, 3, 4 and 5 %). Results in Table 3 indicated that the cell dry weight of *S.cerevisiae* SCC produced after 24 h incubation at 30 C decreased as NaCl concentration increased in the medium. The maximum decrease was observed at 4 % NaCl resulting 87.2 % reduction as compared with control. While, 5 % NaCl gave drastic effect on *S.cerevisiae* SCC growth. The highest growth in presence of NaCl was observed at 1 % NaCl (4.10 g/l) followed by medium containing 2 % NaCl being 2.7 g/l.

On the other hand, glycerol and gassing power were increased by increasing NaCl concentration in growth medium and reached the maximum of 220 mg/g cell dry weigh and 44 cm^3 at 2 % NaCl, respectively. While over this concentration the glycerol content and gassing power decreased again. Also, the reduced glutathione content increased as NaCl content decreased and recorded the highest values at 1 % NaCl being 18 mg/gcell dry weigh. So, it can be stated that the growth of *S.cerevisiae* SCC in presence of 2 % NaCl improved content of glutathione by 30.76 % when compared with control.

In a similar study, Polona, Petra and Peter [15] exposed yeast *S.cerevisiae* ZIM 2155 to NaCl in concentrations of 1-8% (w/v). They found that reactive oxygen increased in cells exposed to 6, 7 and 8% NaCl for 1 h (1.3-fold, 1.9-fold and 2.8-fold increase, respectively) which led to elevate glutathione content in reduced form being 119.1%, 122.6%, 141.5%, respectively.

Effect of Hyperosmotic Stress on Level of Glutathione and Glycerol: Data in Table 4 showed that the glutathione (GSH) content of yeast biomass gradually increased during 60 min of incubation in a medium containing 10 % glucose, while, it decreased in a medium containing 20 or 30 % glucose as compared to control. The highest values of GSH content was recorded after 120 min in a medium containing 10 % glucose followed by 20 % being 35.32 mg/g dry cell (2.39 fold) and 26.0 mg/g dry cell (1.97 fold), respectively. Increasing the incubation time more than 120 min led to slight decrease of GSH content at these concentrations of glucose except in a medium containing 30 % glucose, reached the maximum content after 180 min and then decreased.

On the contrary, the glycerol content Table 5 in all concentrations was sharply increased during the first 30 min of incubation. The glycerol content was approximately constant during the next 30 min in a

Table 6: Effect of hyperosmotic stress on gassing power on *S.cerevisiae* SCC cultured on molasses medium in a fermenter at 30°C as batch culture

	Gassing power(cm^3) content at different glucose concentrations			
Incubation time (min)	0 % glucose	10 % glucose	20 % glucose	30 % glucose
30	38 ± 2.0	49 ± 1.0	66 ± 2.0	40 ± 2.0
60	38 ± 1.0	49 ± 1.5	47 ± 1.3	71 ± 1.0
90	37 ± 2.1	39 ± 1.6	40 ± 2.12	37 ± 1.15

medium containing 10 % (202 mg/gcell dry weigh) or 20 % glucose (220 mg/g dry cell) and then decreased with increasing the incubation time than 60 min. The maximum glycerol content was recorded at 90 min in medium containing 30 % glucose being 230 mg/g dry cell.

Increasing the GSH and glycerol content in yeast cells when exposed to osmotic potential may be due to the yeast cells accumulated the substances to reduce the bad effect of osmotic potential and equivalent the unbalance between the media and inside the cells [16] found that glycerol is the major compatible solute of yeast. In addition; glycerol metabolism plays an important part in redox-balancing.

Data presented in Table 6 showed the high values of gassing power recorded at 20 and 30 % of glucose being 66 cm^3 after 30 min or 71 cm^3 after 60 min incubation, respectively. The increasing gassing power may be due to the high content of intracellular glycerol. This result was in agreement with Hirasawa and Yokoigawa [17], they found high correlation between the intracellular glycerol content and fermentation ability after the osmotic treatment suggested that glycerol accumulated during the hyperosmotic treatment was used in the subsequent fermentation as a substrate and consequently enhanced the fermentation ability.

This treatment improved the GSH content (antioxidant), gassing power and glycerol content of yeast cells which can extend the time of storage of yeast cells when stored as dough [18].

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