The Journal of Microbiology, March 2004, p.51-55
Vol. 42, No. 1

Optimal Fermentation Conditions for Enhanced Glutathione Production by *Saccharomyces cerevisiae* FF-8

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(Received November 12, 2003 / Accepted February 19, 2004)

The influence of feedstock amino acids, salt, carbon and nitrogen sources on glutathione production by *Saccharomyces cerevisiae* FF-8 was investigated. Glucose, yeast extract, KH_2PO_4, and L-cysteine were found to be suitable feedstock. Highest glutathione production was obtained after cultivation with shaking for 72 h in a medium containing glucose 3.0% (w/v), yeast extract 3.0%, KH_2PO_4 0.06% and L-cysteine 0.06%. The glutathione concentration achieved using this medium increased 2.27-fold to 204 mg/l compared to YM basal medium.

Key words: L-cysteine, glucose, glutathione, *Saccharomyces cerevisiae*, yeast

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine) is a well known thiol-containing tripeptide in animals, plants, and microorganisms (Meister *et al.*, 1976). Glutathione is an important component in the cellular mechanisms that protect against UV (Sollod *et al.*, 1992), heavy metals (Perego *et al.*, 1997), and many exogenous organic substances (Goto *et al.*, 1995). Glutathione also plays a sacrificial defense role against oxidative damage in organisms (Berhane *et al.*, 1994). It is now widely used as a medicine, and in health foods to prevent hepatotoxicity induced by acetaminophen, vinyl ethers, or bromobenzene in animals and in the cosmetic industry. The commercial demand for glutathione is expanding. Recently several studies have described glutathione producing yeast strains, which are commonly used for commercial glutathione production (Sakato, 1992; Wei *et al.*, 2003a; Wei *et al.*, 2003b). Many studies have tried to improve the glutathione production by supplementing certain materials, such as glucose, minerals, ATP, and phosphorus to cultures (Li *et al.*, 1998). Amino acids in feedstock are important in the production of glutathione by yeast fermentation (Li *et al.*, 1998b; Alfafara *et al.*, 1992). A glutathione-producing strain, *Saccharomyces cerevisiae* FF-8, was solated from Korean Traditional Rice Wine (Park *et al.*, 2003). In this study, effects of supplementing cultures with precursor amino acids, salts, carbon and nitrogen sources upon the yield and the productivity of glutathione from the isolated *Saccharomyces cerevisiae* FF-8 were investigated in detail.

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Materials and Methods

Microorganism and growth

Saccharomyces cerevisiae FF-8, a glutathione producing yeast strain, was established in our laboratory (Park *et al.*, 2003). FF-8 was aerobically grown in 500 ml flask containing 100 ml of YM medium consisting of 1.0% glucose, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract, at pH 6.0 for 24 h at 30°C. Culture cells were inoculated into 1 L flasks, each containing 200 ml of the same medium and then incubated at 30°C for 72 h with agitation at 100 rpm. After incubation, the culture was centrifuged at 7,000 g for 15 min, the supernatant removed, and the yeast cells washed with distilled water three times. The harvested yeast cells analyzed for glutathione concentration and dry cell weight.

Glutathione analysis and cell growth

The harvested yeast cells were suspended in 0.2 M phosphate buffer (pH 7.2) and disrupted by sonication. The disrupted cells were then removed by centrifugation, and the glutathione concentration in the supernatant was measured using published methods (Cohn *et al.*, 1966), by measuring the absorbance of reaction solutions at 412 nm using a spectrophotometer (UV mini 1240, Shimadzu, Japan). A standard curve generated with known amounts of glutathione was used to determine specimen concentrations. Cell growth was determined by measuring the DCW (dry cell weight). DCW was measured after drying the wet cells at 105°C to constant weight.

Glutathione production conditions

Various carbon, nitrogen, salt, and amino acid sources were tested to determine the best components for glutathione production and cell growth. The most important components were then chosen to optimize concentrations of each component.

Results and Discussion

Effects of various carbon sources

Yeast strains such as *S. cerevisiae* and *Candida utilis* have been reported to produce glutathione (Wei, 2003; Alfafara *et al.*, 1992). We have previously reported that *S. cerevisiae* FF-8, a glutathione producing strain, was isolated from Korean Traditional Rice Wine. Glutathione production by *S. cerevisiae* FF-8 under optimal culture conditions in YM medium was 90 mg/l. Present study was undertaken to investigate the influence of precursor amino acids, salt, carbon and nitrogen sources on glutathione production. The effects of various carbon sources on glutathione production and cell growth of *S. cerevisiae* FF-8 are shown in Table 1. Glucose was found to be the best carbon source for glutathione production and cell growth. Cell growth was similar but glutathione production was 6.6% higher when glucose was used to the carbon source instead of sucrose. Previous studies have reported that when glucose is used as the carbon source, glutathione production and DCW are at a maximum (Liu *et al.*, 1998b; 1999). When lactose was used, glutathione production and cell growth were significantly lowered by 67.4% and 50.0% as compared to that of glucose. Glucose was used as the carbon source for further experiments. The effects of glucose concentrations on glutathione production and cell growth are shown in Fig. 1. Cell growth increased in parallel with increased glucose concentration, which is consistent with the results of Liu *et al.* (Liu *et al.*, 1999). Glutathione production was highest when the glucose concentration was 3.0%.

Table 1. Effects of various carbon sources on glutathione production and on dry cell weight in *Saccharomyces cerevisiae* FF-8 culture.

Carbon source	Glutathione		Dry Cell Weight	
	(mg/l)	(%)	(g/l)	(%)
Glucose	57.6	100	4.15	100.0
Galactose	54.6	94.8	4.05	97.6
Fructose	52.4	91	4.13	97.6
Lactose	18.8	32.6	2.10	50.0
Maltose	56.6	98.3	4.00	95.2
Sucrose	53.8	93.4	4.23	100.0

The medium contained 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, and the carbon sources were added at a concentration of 1.0% (w/v). (n=3)

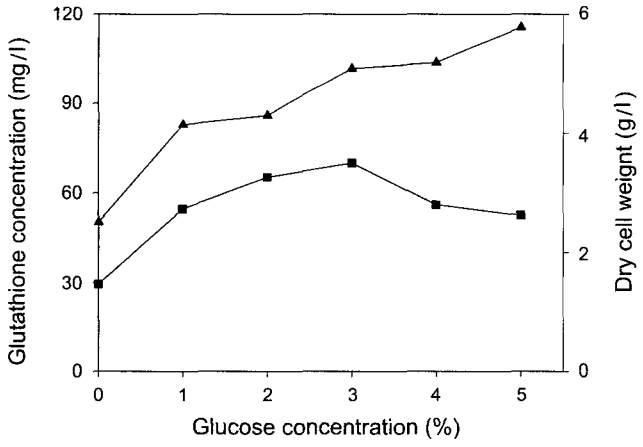

Fig. 1. Effect of glucose concentration on glutathione production and on dry cell weight in *Saccharomyces cerevisiae* FF-8 culture (-■- : Glutathione, -▲- : Dry Cell Weight) (n=3).

Effects of various nitrogen sources

The effects of nitrogen sources on glutathione production and cell growth are shown in Table 2. The medium con-

Table 2. Effect of various nitrogen sources on glutathione production medium and on dry cell weight in *Saccharomyces cerevisiae* FF-8 culture.

Nitrogen source	Glutathione		Dry Cell Weight	
	(mg/l)	(%)	(g/l)	(%)
Peptone	66.4	100.0	4.60	100.0
Tryptone	67.4	101.5	5.80	126.1
Yeast extract	88.4	133.1	5.63	121.7
Malt extract	46.2	69.6	4.85	106.5
Beef extract	44.4	66.9	3.77	82.6
Casein	36.0	54.2	3.94	84.8
Soybean meal	64.2	96.7	4.53	97.8
$NaNO_3$	41.2	62.0	4.28	93.5
NH_4Cl	37.2	56.0	3.83	82.6
$(NH_4)_2SO_4$	26.2	39.5	2.91	63.0

The medium contained 3.0% glucose and the nitrogen sources were added at a concentration of 1.0% (w/v). (n=3)

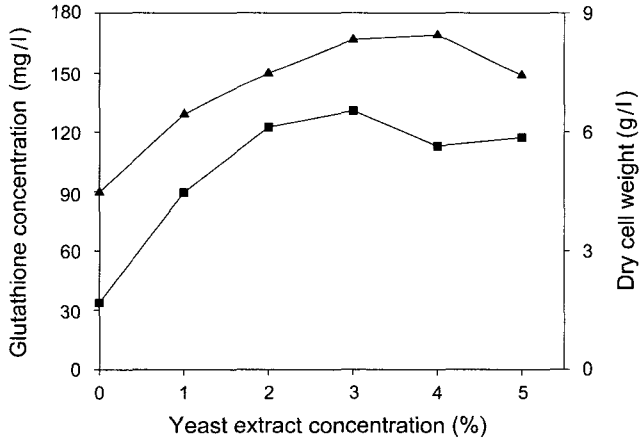

Fig. 2. Effect of yeast extract concentration on glutathione production and on dry cell weight in *Saccharomyces cerevisiae* FF-8 culture (-■- : Glutathione, -▲- : Dry Cell Weight) (n=3).

tained 3.0% glucose, and the various nitrogen sources were supplemented separately at 1.0% (w/v). Yeast extract and tryptone were the best nitrogen sources for glutathione production and cell growth by *S. cerevisiae* FF-8. Previous studies have reported that yeast extract is the best nitrogen source for glutathione production and cell growth (Liu *et al.*, 1999; Shin *et al.*, 1993). Thus, yeast extract was chosen as the nitrogen source for further experiments. Glutathione production and cell growth were highest when the concentrations of yeast extract were 3.0% and 4.0%, respectively (Fig. 2). Previous studies have also reported that the highest glutathione production of *Candida* sp. was obtained in culture media containing yeast extract 4.0% as a nitrogen source (Kim *et al.*, 1993; Shin *et al.*, 1993).

Effects of various salt sources

The effects of various salt sources on glutathione production and cell growth are shown in Table 3. In order to identify the effect of salts, the medium contained 3.0% glucose and 3.0% yeast extract, and the salts were separately supplemented at 0.05% (w/v). All salts except for $FeSO_4$ stimulated glutathione production and cell growth as compared to salt free medium. As KH_2PO_4 was found to be the best salt source in terms of glutathione production and cell growth, it was chosen for further study. KH_2PO_4 at a concentration of 0.06% was found to be sufficient for *S. cerevisiae* cell growth and glutathione production (Fig. 3).

Effects of various amino acid sources

The effects of various amino acid sources on glutathione production and cell growth are shown in Table 4. The medium in this experiment contained 3.0% glucose, 3.0% yeast extract and 0.06% KH_2PO_4; amino acid sources were supplemented at 0.05% (w/v). Li *et al.* reported that the DCW and the intracellular glutathione content increased when 2.0 g/l ATP and 9 mmol/l precursor amino acids were added at the beginning of fermentation by 24% and 1.4-fold, respectively, compared to fermentation without these additions (Li *et al.*, 1998a; Li *et al.*,

Table 3. Effect of various salt sources on glutathione production and on dry cell weight in *Saccharomyces cerevisiae* FF-8 culture.

Salt source	Glutathione		Dry Cell Weight	
	(mg/l)	(%)	(g/l)	(%)
Control[a]	127.0	100.0	8.30	100.0
$MgSO_4$	135.8	106.9	8.30	100.0
K_2HPO_4	140.0	110.2	8.60	103.6
KH_2PO_4	144.8	114.0	8.68	104.8
NaCl	130.2	102.5	8.73	104.8
$CaCl_2$	128.6	101.3	8.73	104.8
$FeSO_4$	126.4	99.5	8.53	102.4
$ZnSO_4$	143.2	112.8	8.85	107.2
$MnSO_4$	140.0	110.2	8.73	104.8

[a]The control medium contained 3.0% glucose, 3.0% yeast extract, and the salts were added at a concentration of 0.05% (w/v). (n=3)

Table 4. Effect of various amino acid on glutathione production and on dry cell weight in *Saccharomyces cerevisiae* FF-8 culture.

Amino acid source	Glutathione		Dry Cell Weight	
	(mg/l)	(%)	(g/l)	(%)
Control[a]	158.0	100.0	8.40	100.0
L-Glutamic acid	139.0	88.0	8.15	97.6
L-Cysteine	200.4	126.8	8.28	98.8
Glycine	132.8	84.1	7.43	88.1
L-Methionine	150.0	94.9	7.98	92.9
L-Cystine	140.6	88.9	8.40	100.0
Taurin	134.0	84.8	7.90	94.0

[a]The control medium contained 3.0% glucose, 3.0% yeast extract, KH_2PO_4 0.06%, and the amino acid sources were added at a concen tration of 0.05% (w/v). (n=3)

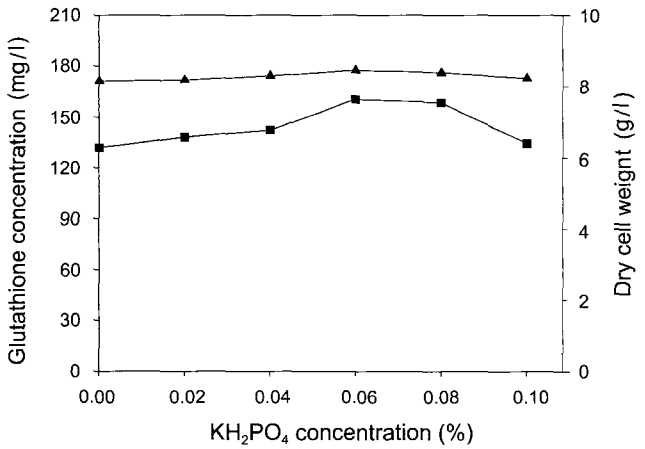

Fig. 3. Effect of KH_2PO_4 concentration on glutathione production and on dry cell weight in *Saccharomyces cerevisiae* FF-8 culture (-■- : Glutathione, -▲- : Dry Cell Weight) (n=3).

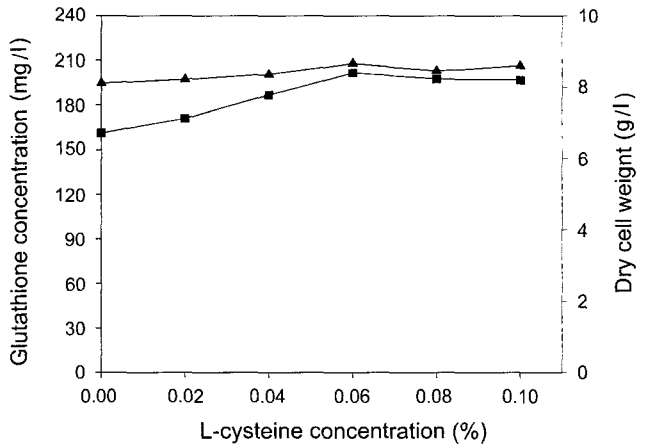

Fig. 4. Effect of L-cysteine concentration on glutathione production and on dry cell weight in *Saccharomyces cerevisiae* FF-8 culture. (-■- : Glutathione, -▲- : Dry Cell Weight) (n=3)

Table 5. Optimal medium compositions for glutathione production by *Saccharomyces cerevisiae* FF-8.

Medium component	Concentration (w/v)
Glucose	3.0%
Yeast extract	3.0%
KH_2PO_4	0.06%
L-Cysteine	0.06%

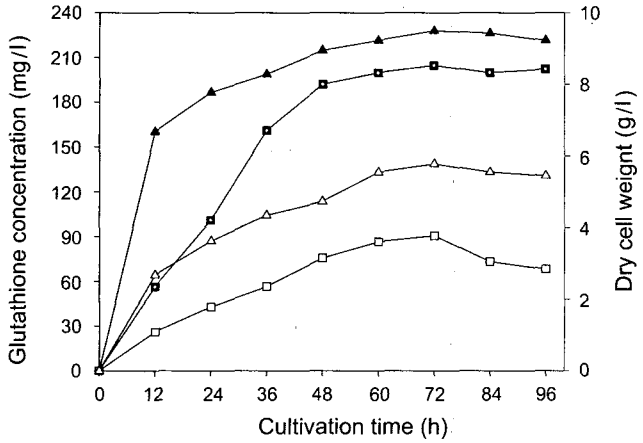

Fig. 5. Time courses of glutathione production in medium and in dry cell weight in *Saccharomyces cerevisiae* FF-8 culture in the optimal medium and in YM medium. Optimal medium (-□- : Glutathione, -△- : Dry Cell Weight), YM medium (-■-: Glutathione, -▲-: Dry Cell Weight) (n=3).

1999). This result suggested that the addition of precursor amino acids and ATP could promote intracellular glutathione accumulation. L-cysteine, a precursor amino acid of glutathione, was found to be the best amino acid for glutathione production, and chosen for further study. Glutathione production and cell growth were highest when the concentration of L-cysteine was 0.06%, beyond which it decreased (Fig. 4).

From these results, the best medium composition for glutathione production by *S. cerevisiae* FF-8 was; 3.0% glucose as carbon source, 3.0% yeast extract as nitrogen source, 0.06% KH_2PO_4 as salt source, and 0.06% L-cysteine as precursor amino acid of glutathione (Table 5). The glutathione concentration reached 204 mg/l using this medium (Fig. 5), which was higher than the 64.7 mg/l obtained by cultivating *S. cerevisiae* for 32 h by Wei *et al.*, the 175 mg/l of *Candida* sp by Shin *et al.*, and the 119.4 mg/l of yeast by Li *et al.*, (Wei *et al.*, 2003; Shin *et al.*, 1993; Li *et al.*, 1998b).

In conclusion, The glutathione concentration produced by *S. cerevisiae* FF-8 using this medium significantly increased by 2.27-fold compared to the 90 mg/l achieved in YM medium.

Acknowledgment

This study was supported by the Dong-A University Research Fund, in 2003.

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