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Yeast selection for high resistance to and uptake of Se: Cultural optimization of organic selenium production

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Se-rich yeast within animal feeds is much more effective than additions of inorganic Se in increasing the concentration of Se in eggs, milk and meat. This study was conducted in order to select mutant *Saccharomyces cerevisiae* which produce higher levels of organic selenium (Se) and to improve the productivity of this Se-rich yeast by optimization of the culturing condition. Among 13 ATCC strains of *S. cerevisiae*, ATCC 560 showed a higher tolerance towards Se, exhibiting a total Se uptake rate of 6.69 mg/l. The mutant *S. cerevisiae* 6M, which is an ATCC 560 derivative developed through UV mutagenesis, showed about 20% increased Se production rates (8.0 mg/l). Optimal culturing conditions were determined, in terms of the timing and addition of inorganic Se, initial pH, and overall culturing time. The optimal concentration of inorganic Se was determined to be 125 ppm, the optimum time for the addition of which was determined to be at the start of incubation. The optimal initial pH of the medium and culturing time was 6.0 and 9 h, respectively. Under these conditions, *S. cerevisiae* 6M showed a total Se production of 10.87 mg/l, a 63% increase compared to that of with ATCC 560 under normal culture conditions.

Key words: *Saccharomyces cerevisiae*, organic selenium, UV mutagenesis, culture condition.

INTRODUCTION

Organically-bound Se is essential for the growth of animals and humans alike. Se is an important element found in selenoproteins and enzymes with various physiological functions, such as antioxidant defense, inflammation reduction, thyroid hormone production, DNA synthesis, fertility, and reproduction (Rayman, 2000). Critically, Selenized yeast treatments delivering 200 µg/day of Se were found to decreases total cancer incidence (Clark and Marshall, 2001; Reid et al., 2008).

Milk, meat, chicken, fish, and eggs are protein-rich foods, containing high levels of Se (Klapec et al., 2004; McNaughton and Marks, 2002; Pappa et al., 2006; Sirichakwal et al., 2005). The ingestion of Se-fortified poultry and pork meats through recommended quantities is a safe and natural way to increase the daily intake of Se-methionine (Olivera et al., 2005). Organic Se supplements, such as those sourced from Se yeast, are much more effective than those of inorganic Se in increasing the concentration of Se in egg, milk, blood, and plasma (Fisinin et al., 2008; Ortman and Pehrson, 1999; Slavik et al., 2008). Organic Se is a highly bioavailable form of Se for chickens and other livestock, and provides a greater level of antioxidant protection than inorganic Se (Mahan, 1999; Mahmoud and Edens, 2003).

The Se content of muscle is also higher in animals fed Se yeast when compared to applications of similar doses of sodium selenite (Juniper et al., 2009; Vignola et al., 2009). Se yeast has been shown to enhance meat quality (Edens, 1996; Mahan et al., 1999), growth of feathers (Edens, 1996), and positively influence the thyroxine conversion to tri-iodothyronine and the passive immunity of newborn lambs (Rock et al., 2001). It is known that in some microorganism, especially yeasts, large amount of Se can be incorporated into cellular proteins, mainly in the form of selenomethionine, the best source of organic Se (Demirci and Pometto, 1999). Kelly and Power (1995)

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Table 1. Viability of the wild-type *S. cerevisiae* strains cultured in YM medium containing different concentrations of Se.

Strain		Se concentration (ppm)						
ATCC No.	Origin	0	50	125	250	500	1,000	1,500
		cfu/ml						
560	Distillery yeast	3.3×10^7	3.1×10^6	9.0×10^6	6.8×10^6	7.4×10^6	ND[1]	ND
2341	Holly berries	1.6×10^7	2.7×10^7	1.8×10^7	2.9×10^5	ND	ND	ND
4123	Burgundy wine yeast	2.8×10^7	1.3×10^7	3.7×10^6	ND	ND	ND	ND
4126	Amylo process yeast	4.1×10^7	ND	ND	ND	ND	ND	ND
6037	Baker's yeast	1.2×10^7	4.3×10^6	7.6×10^6	8.9×10^5	ND	ND	ND
10604	Billi Wine yeast	2.7×10^7	1.0×10^6	3.0×10^5	4.9×10^6	ND	ND	ND
13668	Fermented grape juice	2.8×10^7	1.2×10^7	1.8×10^7	3.2×10^6	ND	ND	ND
24858	Unknown	3.1×10^7	1.1×10^6	2.0×10^5	3.1×10^6	1.3×10^6	ND[1]	ND
24903	Yeast cake	2.1×10^7	1.7×10^7	2.5×10^7	4.9×10^6	2.1×10^6	ND	ND
26422	Sake yeast	5.1×10^7	ND	ND	ND	ND	ND	ND
26787	Unknown	5.1×10^7	2.1×10^7	2.7×10^7	ND	ND	ND	ND
42507	Fermenting grape must	3.1×10^7	2.2×10^7	2.6×10^7	3.7×10^6	ND	ND	ND
56478	Wine yeast	1.6×10^7	5.0×10^6	8.8×10^6	1.8×10^4	4.8×10^3	9.7×10^2	4.4×10^3

[1]Not detected.

demonstrated that approximately 94% of the Se in the Se-enriched yeast source was organically incorporated into one of several seleno-amino acid analogs, the major one being selenomethionine. 85% of Se contained in Se yeast was found to be present in the form of Se-methionine and 91% was organic (Ip et al., 2000; Fan et al., 2003). As Se-enriched probiotics, yeast strains such as *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, and *Candida utilis* were reported (Pan et al., 2011; Sara et al., 2006; Suhajda et al., 2000). The purpose of the present work was to screen for higher production strains of Se yeast. *S. cerevisiae* (ATCC 560) was selected for its having the highest tolerance of Se, and was mutated through UV irradiation. Finally, the culture conditions of the mutant *S. cerevisiae* 6M were optimized in order to maximize the Se content.

MATERIALS AND METHODS

Yeast strains and culture conditions

The 13 strains of *S. cerevisiae* used in this study, as well as pertinent information such as their origins are described in Table 1. To evaluate the resistance of the yeast strains to high concentrations of Se, yeast mold (YM) agar plates (10 g/l glucose, 3 g/l yeast extract, 3 g/l malt extract, 5 g/l peptone and 1.5% agar) were used with various concentrations of inorganic Se in the form of sodium selenite. The cultures of *S. cerevisiae* were prepared by incubation in YM broth at 30°C with agitation (180 rpm) for 24 h. Each seed culture (60 ml) was mixed with the feeding medium including sodium selenite (30 ml) in an Erlenmeyer flask.

Determination of cellular Se concentration, dry cell weight and total Se uptake rates

Se was determined according to the Association of Official Analytical Chemists (AOAC) Official Method 2006.03 (Kane and Hall, 2006) with some modifications. An *S. cerevisiae* culture (10 ml) was centrifuged (10,000 rpm for 10 min at 4°C) to harvest the cells. The cell mass was washed twice with distilled water. Next, 10 ml of HNO_3 was added to the harvested cells and the mixture was heated in a microwave (MARS, CEM Co., USA) to disrupt and digest the cells. The ramp temperature was increased from room temperature to 200°C over 15 min, and the holding time at 200°C was 20 min. The Se concentration in disrupted cells was determined by using an inductively coupled plasma-optical emission spectrometer (ICP-OES; Varian VISTA-PRO, USA). The dry cell weight had been determined by separating the cells from the broth after centrifugation at 10,000 rpm for 10 min, washing the cell mass three times with distilled water, and drying the cells at 70°C until a constant dry weight was obtained. The total Se uptake rate was calculated by multiplying the dry cell weight with the ICP-OES determined cellular Se concentration.

UV mutagenesis and mutant characteristics

The *S. cerevisiae* ATCC 560 broth culture was mixed with YM broth at a 1:1 ratio and exposed to a ultraviolet (UV) lamp (6 W, 254 nm; Sankyo Denki, Japan) at a distance of 30 cm. After UV-mutagenesis, the mixture was spread onto YM agar plates containing 2,000 mg/l Se and incubated for 2 days at 30°C. Colonies were then isolated and used for further studies. Seven different Se concentrations (0, 31, 63, 125, 250, 500 and 1,000 ppm) were applied to the medium in order to determine the optimum concentrations which would yield maximum Se uptake in the mutant *S. cerevisiae* 6M strain. The mutant strain was also tested for optimal time of Se addition, harvesting time of yeast cells, and the effect of initial pH in media for maximizing Se uptake.

RESULTS AND DISCUSSION

Se tolerance and uptake in wild-type *S. cerevisiae*

To identify the strain of *S. cerevisiae* with the highest Se tolerance, yeast strains were cultured in a medium

containing various concentrations of Se (Table 1). The growth of most yeast strains was inhibited with increased concentration of Se in the medium. Two strains, ATCC 4126 and ATCC 26422, exhibited no growth when the Se concentration exceeded 50 ppm. All yeast strains, except for four (ATCC 560, ATCC 24858, ATCC 24903 and ATCC 56478), exhibited no growth in the presence of 500 ppm Se. ATCC 560 exhibited the highest cell count (7.4×10^6 cfu/ml) at 500 ppm Se, and ATCC 56478 grew even in the highest concentration of Se (1,500 ppm). A similar inhibitory effect of increased Se concentrations was also reported by Golubev and Golubev (2002). They tested the Se tolerance of yeast using Se concentrations ranging from 0 to 7,900 ppm, where a Se concentration of 790 ppm inhibited the growth of most of the strains on a glucose-peptone agar.

The efficiency of Se uptake in various wild strains of yeast was examined by adding 125 ppm Se. The ATCC 4126 strain yielded the highest dry cell weight, 1.83 g/l (Figure 1A). However, the cellular Se concentration, 2.25 mg/g of dry cell weight (Figure 1B) and total Se uptake rate, 4.11 mg/l (Figure 1C) was low. Although the ATCC 56478 strain showed the highest tolerance towards Se in the medium, the dry cell weights and cellular Se concentrations lower than all other yeast strains. The highest rate of total Se uptake was observed in the ATCC 560 strain (6.69 mg/l), which also showed the highest cell count at different Se concentrations, and even up to 500 ppm, as shown in Table 1. Although the tested strains were the same species of *S. cerevisiae*, the dry cell weight and Se uptake efficiency varied. The observed range of dry cell weight was 1.3 to 1.83 g/l, and the cellular Se concentrations were 1.29 to 3.98 mg/g of dry cell weight. In this study, the uptake rates of the ATCC 560 strain were higher than those of other strains reported previously. The cellular Se concentration of the *S. cerevisiae* ATCC 560 strain is about 2.1 times higher compared to *S. cerevisiae* ATCC 26787, reported by Demirci and Pometto (1999) and is 1.7 times higher compared to *S. cerevisiae* reported by Ponce et al. (2002). *S. cerevisiae* ATCC 560, showing the highest cellular Se concentrations and total Se uptake rates of all strains, was selected for further experiments.

Isolation of mutant strains showing enhanced Se uptake

S. cerevisiae ATCC 560 were mutated by UV radiation to improve Se uptake efficiency. After mutation, the strains showing a tolerance in a media containing 2,000 ppm Se were selected. Dry cell weight, cellular Se concentration, and total Se uptake rates were compared. Some of the mutant strains showed higher dry cell weights and total Se productions than the parent strain. *S. cerevisiae* 6M selected among eight selected mutants had the highest cellular Se concentration (4.55 mg/g of dry cell weight) and total Se uptake rate (8.0 mg/l) compared to parent strain. *S. cerevisiae* 6M showed a 20% increase in total Se uptake.

Effect of Se concentration on *S. cerevisiae* 6M

The effect of different Se concentrations on dry cell weight, cellular Se concentration, and total Se uptake rates are shown on Table 2. When Se was not added to the culture medium, dry cell weight was the highest (3.15 g/l); this weight was lowest (1.21 g/l) at 1000 ppm Se. Decreased dry cell weight associated with increasing concentrations of Se indicated that higher concentrations of Se inhibit cell growth. The addition of 31 to 125 ppm Se to the medium led to elevated cellular Se concentrations of between 1.39 and 5.03 mg/g of dry cell weight. However, the addition of more than 250 ppm of Se to the medium caused dry cell weight and total Se uptake rates to decrease. The highest dry cell weight (5.03 mg/g) and total Se uptake rate (8.24 mg/l) were observed in the presence of 125 ppm Se.

These results are similar to ones from studies of Se-resistant wild-type strains. The viability of most strains decreased, or strains died, in the presence of 250 ppm Se, and the viability did not change in the presence of 125 ppm (Table 1). Therefore, the highest Se concentration that did not inhibit biomass production was 125 ppm. In this study, the optimal concentration for Se-rich yeast was 125 ppm even though *S. cerevisiae* 6M was identified under conditions of 2,000 ppm Se.

A culture medium supplemented with 30 ppm Se, as sodium selenite, during the exponential growth phase resulted in a Se accumulation of 1.2 to 1.4 mg/g in *S. cerevisiae*, as measured by the previously described ICP-AES method (Suhajda et al., 2000). The Se content in yeast cells was found to be significantly increased from 4.76 to 8.69 µg/g, with increasing concentrations of Se (1.5 to 4.5 ppm) in the medium (Kaur and Bansal, 2006). Another group observed that yeast cultivated in media supplemented with different concentrations of Se (2 to 12 ppm) contained 15 to 203 µg Se/g of dry cell weight (Stabnikova et al., 2008).

Optimal time for Se addition and harvesting of yeast cell

To improve Se uptake efficiency, the timing of Se additions, and harvesting of the cultures was investigated (Figure 2). When the Se concentration in the culture medium was adjusted to 125 ppm, the dry cell weight of *S. cerevisiae* 6M was found to increase as the timing of Se additions were delayed. Dry cell weight was the highest when Se was added after 9 h and cell harvesting time was 10 and 24 h, showing 2.58 and 2.34 g/l, respectively.

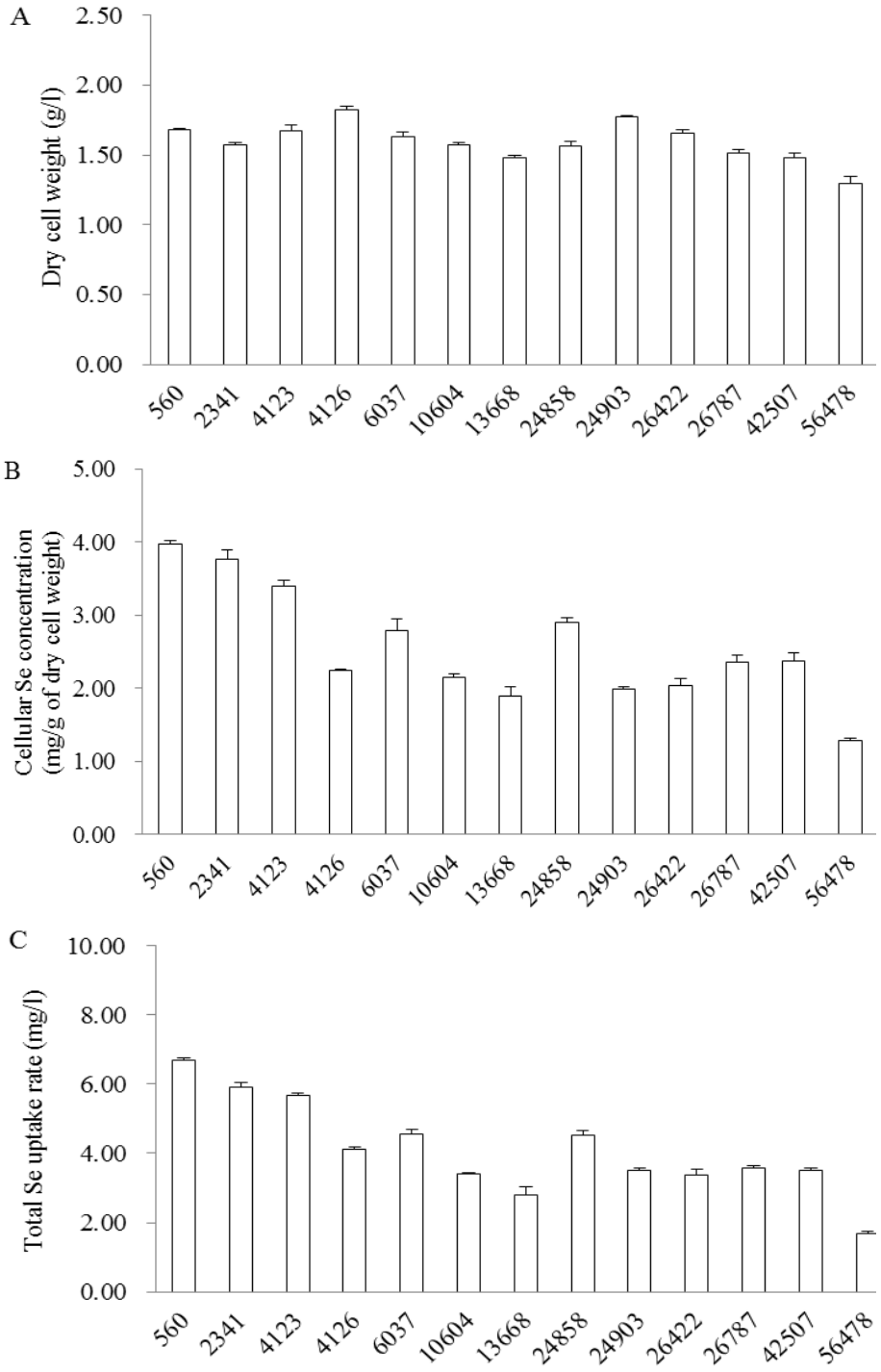

Figure 1. Dry cell weight (A), cellular Se concentration (B), and total Se uptake rate (C) from different strains of *S. cerevisiae* in YM medium supplemented with 125 ppm Se. The number is the ATCC No. assigned to each strain of *S. cerevisiae*.

When Se was added at the starting point (0 h) of the culture, the cellular Se concentrations after 10 and 24 h of incubation were the highest at 5.73 and 5.13 mg/g of dry cell weight, respectively. Cellular Se concentrations were significantly decreased when Se was added at a later time. The total uptake rate of Se was the highest, at 9.83 and 8.20 mg/l incubated at 10 and 24 h, respectively. Cell age can also influence metal

Table 2. Effects of different Se concentrations on dry cell weight, cellular Se concentration, and total Se uptake in mutant *S. cerevisiae* 6M.

Se addition level (ppm)	Dry cell weight (g/l)	Cellular Se concentration (mg/g of dry cell weight)	Total Se uptake rate (mg/l)
0	3.15±0.05[1]	ND[2]	ND
31	1.69±0.02	1.39±0.02	2.36±0.03
63	1.66±0.06	3.44±0.08	5.72±0.14
125	1.64±0.02	5.03±0.06	8.24±0.09
250	1.43±0.06	4.51±0.05	6.45±0.06
500	1.25±0.03	1.38±0.03	1.72±0.03
1000	1.21±0.00	0.61±0.03	0.74±0.03

[1]Data are represented as the mean±standard deviation. [2]Not detected.

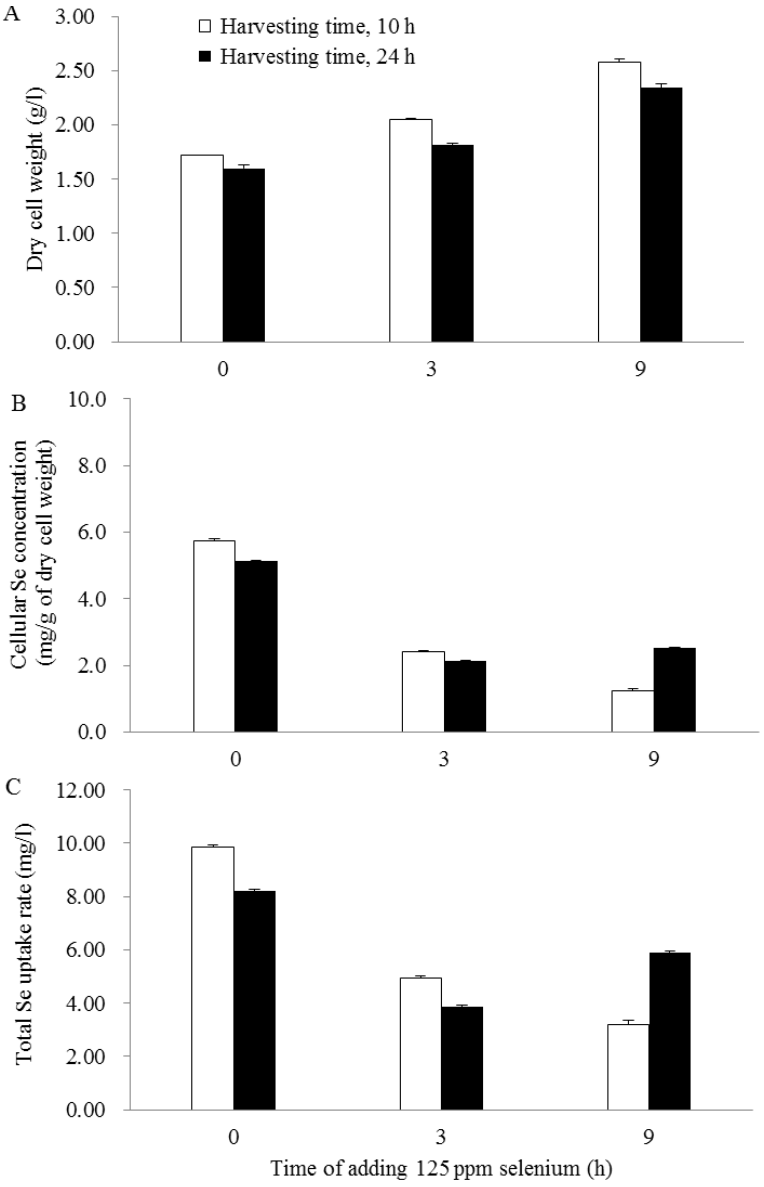

Figure 2. Effects of different Se addition times and cell harvesting times on dry cell weight **(A)**, cellular Se concentration **(B)**, and total Se uptake rate **(C)** of mutant *S. cerevisiae* 6M.

Table 3. Effects on initial pH on dry cell weight, cellular Se concentration, and total Se uptake of *S. cerevisiae* 6M mutant strains cultured in YM medium containing 125 ppm of Se.

Initial pH	Dry cell weight (g/l)		Cellular Se concentration (mg/g of dry cell weight)		Total Se uptake rate (mg/l)	
	Wild-type[1]	Mutant	Wild-type	Mutant	Wild-type	Mutant
3.0	1.12±0.00[2]	1.31±0.00	4.61±0.06	5.15±0.06	5.15±0.07	6.77±0.07
4.0	1.30±0.01	1.48±0.01	5.67±0.13	5.55±0.01	7.38±0.16	8.19±0.02
5.0	1.36±0.00	1.57±0.01	5.80±0.05	5.91±0.01	7.91±0.07	9.31±0.01
6.0	1.48±0.01	1.70±0.01	4.63±0.03	6.40±0.04	6.84±0.05	10.87±0.06
7.0	1.49±0.00	1.66±0.02	2.53±0.02	3.54±0.03	3.77±0.03	5.87±0.05

[1]Wild-type strain was *S. cerevisiae* ATCC 560. [2]Data are represented as the mean±standard deviation.

biosorption (Goyal et al., 2003). Usually, the cells in the lag phase or early stages of growth have a higher biosorption capacity for metal ions than that those in the stationary phase (Kapoor and Viraraghavan, 1997).

Various harvesting times were further investigated to optimize Se uptake (data not shown). As a result, total Se uptake rate was highest (10.32 mg/l) when cells were incubated for 9 h. Incubation longer than 9 h caused Se uptake rates to decrease. A similar trend was observed in a previous study of *S. cerevisiae* cells enriched with copper. The maximum amounts of copper uptake were obtained after 8 h of incubation (Mrvcic et al., 2007).

Effect of initial pH on Se-rich yeast

The effects of initial pH on the *S. cerevisiae* 6M mutant strain is shown in Table 3. The concentration of Se in the media (125 ppm), time when the Se was added (0 h), and cell harvesting time (9 h) were fixed so that Se uptake efficiency was optimized. The dry cell weight of the wild-type strains was highest when initial pH was 6.0, or 7.0. The dry cell weight of the mutant strain was the highest (1.7 g/l) with an initial pH 6.0. Decreasing the pH of the medium tended to reduce dry cell weight. The dry cell weights of wild and mutant strains were the lowest (1.12 and 1.31 g/l, respectively) at an initial pH 3.0. In the *S. cerevisiae* 6M mutant, an initial pH 6.0 resulted in the highest cellular Se concentration (6.4 mg/g of dry cell weight) and Se production rate (10.87 mg/l), respectively. In *S. cerevisiae,* the optimal pH value for copper biosorption has been reported as 5 to 9, while it was 4 to 5 for uranium biosorption (Volesky, 1990).

In conclusion, the mutant *S. cerevisiae* 6M strain was screened by UV radiation and showed a 20% increase in Se uptake compared to the parent strain within this study. Culture conditions were optimized to improve Se uptake rates. Under the optimal conditions (pH 6.0; Se concentration, 125 ppm; adding time of inorganic Se, 0 h; cell harvesting time, 9 h), the total Se uptake rate of *S. cerevisiae* 6M was 10.87 mg/l, showing a 63% increase compared to the parent strain. *S. cerevisiae* 6M is suitable for further development as a feed additive for the production of Se-fortified poultry and pork meats.

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