Short Communication

Acceleration effect of sodium selenite on yeast growth and fermentative capability

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Selenium (Se), one of the 14 kinds of essential trace elements, plays an important role in every field of bio-science. Selenium is a key component of numerous functional selenoproteins which contain glutathione peroxidase (GSH-Px), thioredoxin reductase (TrxR), selenoprotein P and selenoprotein W (Zeng, 2009). GSH-Px protects mammalian systems from oxidative stress and has been related to the prevention of chronic diseases and cancer (Beckett and Arthur, 2005).

Recently, many applications of Se-enriched yeast have been studied; for example, Bakery yeast (*Saccharomyces cerevisiae*) is capable of metabolizing up to 3,000 mg Se/g converting approximately 90% of the selenium into L-SeM and leaving traces of the inorganic form (California Environmental Protection Agency, 2010). The advantage of such bread is that, without an extensive modification of the process, it can yield significant amounts of organic selenium and be mass produced. Additionally, selenium introduced with Se-enriched sprouted seeds would reveal a much higher bioavailability than inorganic supplements (Fairweather-Tait, 1997; Schrauzer, 2000).

As in the case of humans, animals, and plants, the metabolism and growth of yeast also requires selenium, and there should be an optimum quantity for this. Although there have been various research works about the applications of se-enriched yeast, few attempts have been made to determine the concentration of inorganic selenium which can promote the growth of yeast. Therefore, the present study was undertaken with the purpose of determining the optimum concentration of sodium selenite under which the reproduction of yeast is better and its fermentative capability is also increased, to some degree.

Sodium selenite, the source of the selenium, was of high purity. *Saccharomyces cerevisiae* JS, JN and JW, isolated from brewer's yeast powder, are used widely in selenium enrichment. *Zygosaccharomyces rouxii* F, FM and PC, isolated from soybean sauce and a residue of soya, are tolerant of a high salt concentration. All the stored strains were cultivated according to the procedure described by Shaokui et al. (2005) with minor modifications. Briefly, the culture media (YPD media) were treated at 121°C at 15 psi in an autoclave (Sterilizer SE-510, Yamato, Japan) for 15 min. After that, six stored strains were spread in the cooling sterilized solution with the same inoculation amount and incubated at 28°C in a reciprocal shaker (179 rpm) for 48 h, during which time the concentration of each strain was detected by a spectrophotometric method with a wavelength of 600 nm every four hours. In order to select the strains which have a better reproductive capacity, the yeast growth curve was then drawn according to the absorbance. At this stage, the strains of F and JS were selected to move forward a single step (data not shown), and their salt tolerance was also detected. The comparative result indicated that JS could develop in 10% of salt while F could tolerate 18% of salt (data not shown). This consequence agrees with the source of the two strains and F is suitable for use in pickles.

A series of concentration gradients (0; 0.50; 3.0; 5.0; 8.0; 12; 15; 20; 25; 30; 40; 50 µg sodium selenite/mL nutrient solution) were chosen following the research data of Hart et al. (2011), Liguang et al. (2011) and Penglase et al. (2011), so that a primary range of sodium selenite additive amount could be determined. For this purpose, the selected strains were inoculated in the solid media (PDA

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Fig. 1. Effect of sodium selenite concentration on the growth of yeast.

A (B) shows the growth condition of the F (JS) strain with different concentrations of sodium selenite. The growth condition is measured by cell density.

media). After cultivation in a constant temperature incubator at 28°C for 48 h, the colony morphology of each plate was evaluated. Following this, according to the growth conditions, the exact concentration of sodium selenite was determined with the cultivation in a conical flask. As Fig. 1 shows, when the concentration was increased from 0 to 0.5 μg/mL, the optical density of the F yeast strain rose from 47.05 to 52.32, and that of the JS strain increased from 35.25 to 40.55. However, both were inhibited at an excess of 1.2 μg/mL sodium selenite. This is the first time that this promoting effect on the growth of yeast, in this concentration range, has been reported. The biomass of the JS strain increased by 15.04%, while that of the F strain increased by 11.2%, indicating that the acceleration of 0.5 μg/mL sodium selenite on the growth of *Saccharomyces cerevisiae* is more evident than that of *Zygosaccharomyces rouxii*. Furthermore, with the increase in the inorganic selenium concentration, the JS strain was restrained more slightly than the F strain. A similar experimental phenomenology of high-concentration selenium inhibiting the growth of yeasts was proposed by Hart et al. (2011). Also, the better tolerance of *Saccharomyces cerevisiae* is in agreement with Pérez-Corona et al. (2011) and Hossein et al. (2014). Nowadays, the relative products of se-enriched yeast are also using *Saccharomyces cerevisiae* as the raw material.

In general, the growth cycle of yeast is 48–72 h, however, during the different stages, the rate of growth differs widely, and when the additive is introduced may cause different effects on the growth of the yeast. Therefore, it is necessary to determine the best moment to add the so-

Fig. 2. Effect of sodium selenite addition time on the growth of yeast.

A (B) is the growth condition of the F (JS) strain with different addition times of 0.5 μg/mL sodium selenite. The growth condition is measured by cell density.

dium selenite. YPD culture medium and sodium selenite solution were put into the sterilizing pot individually and mixed at different growth periods of the yeast. The same dose of sodium selenite was added at the different growth stages of the yeast, resulting in different influences on the biomass of the yeast. According to Fig. 2, with the carry-forward of addition time, the change has no obvious regularity, but addition at the twelfth hour leads to the most significant effect. The twelfth hour is also the starting point of the logarithmic phase, during which the trace element Se plays a big role. During the yeast breeding process, selenium element could replace the sulfur element and thereby become involved in the protein composition: mainly, selenomethionine and selenocystine.

Alcohol dehydrogenase (ADH) and alkaline protease are two widely adopted industrial enzymes in the food industry. The effect of Se on the activity of alcohol dehydrogenase and alkaline protease was detected. Alcohol dehydrogenase, an intracellular enzyme, could be detected after yeast cell disruption. Centrifuged yeast cell sediment was dried and disrupted as described by Zanon et al. (2007) with a slight modification, using a 10 mL screw-capped centrifuge tube, 1 mL of 2 mM sodium citrate buffer at pH 6.2 containing 2 mM β-mercaptoethanol, and 2% (w/v) of lywallzyme with a water bath at 30°C for 1 h. The centrifuge tubes with enzymatic hydrolysate were then transferred to an Ultrasonic Cell Disruption System (ultrasonic power: 420 W; ultrasonic time: 11 min; disruption time: 12 min) to become adequately broken. The supernatant separated by centrifugation (10 min at 12,000 rpm in the centrifuge) was used for the alcohol dehydrogenase assays. The alcohol dehydrogenase activity detec-

Fig. 3. Effect of sodium selenite and salt on the ADH activity.

Activities of alcohol dehydrogenase (ADH) on the cytoplasmic membrane of the F and JS strains under different conditions. Control (I) is the common culture condition; I is the condition with 0.5 $\mu\text{g/mL}$ sodium selenite; Control (II) is the condition with 8% salt; II is the condition with 8% salt and 0.5 $\mu\text{g/mL}$ sodium selenite. Three parallel experimental data were processed by a one-way analysis of variance (ANOVA) with a homogeneity test of variance. * refers to a significant difference and ** refers to an extremely significant difference.

Fig. 4. Effect of sodium selenite and salt on the alkaline protease activity.

Activities of alkaline protease on the cytoplasmic membrane of the F and JS strains under different conditions. Control (I) is the common culture condition; I is the condition with 0.5 $\mu\text{g/mL}$ sodium selenite; Control (II) is the condition with 8% salt; II is the condition with 8% salt and 0.5 $\mu\text{g/mL}$ sodium selenite. Three parallel experimental data were processed by a one-way analysis of variance (ANOVA) with a homogeneity test of variance. * refers to a significant difference.

Table 1. Organic selenium conversion calculated in different experiment groups.

Samples	Concentration of inorganic selenium ($\mu\text{g/mL}$)	Organic selenium conversion
Blank culture	0	—
F		
I	0.053 ± 0.001	89.40%
II	0.075 ± 0.002	85.00%
JS		
I	0.034 ± 0.001	93.20%
II	0.086 ± 0.002	82.80%

tion method described by Gokhale et al. (1986) was optimized for the yeast ADH assay. The total reaction mixture of 3.0 mL of supernatant; 1 mL of 1% (w/v) bovine serum albumin and 1.0 mL of 0.1 mol/L phosphate buffer (pH 7.4) was mixed evenly. 0.1 mL was then taken out and mixed evenly with 1.5 mL of 0.1 mol/L sodium phosphate buffer (pH 8.8); 1.0 mL of 2.0 mol/L alcohol and 1.0 mL of 0.025 mol/L NAD. The change in absorbance at 340 nm was monitored every 15 s for one minute. All operations were carried out in triplicate and the average values were adopted to calculate the activity using the following formula:

$$U = E_{340} \times 3.6 / (0.1 \times M),$$

where E_{340} is the mean change in absorbance at 340 nm; 3.6 is the total volume of the reaction liquid; 0.1 is the volume of the enzyme sample; and M is a fixed parameter 0.01. The result is presented in Fig. 3. Compared with the control group, the ADH activity of I group is noticeably higher and a similar result is obtained in the two salt addition groups, even though the extent of the increase is a little less. Three parallel experimental data were processed by a one-way analysis of variance (ANOVA) with a homogeneity test of variance. For the F strain, the addition of 0.5 $\mu\text{g/mL}$ Na_2SeO_3 significantly affected ADH activ-

ity ($p < 0.05$), but 8% salt did not ($p > 0.05$). Whereas, for the JS strain, the addition of 0.5 $\mu\text{g/mL}$ Na_2SeO_3 and 8% salt both significantly affected ADH activity ($p < 0.05$). Because of the JS strain is without salt tolerance, 8% salt would have negative effect on its ADH activity.

As for the effect of Se on the activity of alkaline protease, the preparation of the standard curve and the detection of samples were carried out according to the foline-phenol method (Lowry et al., 1951). Protease is a crucial enzyme for hydrolyzing a variety of proteins, and the hydrolysates (amino acids and polypeptides) have effects on the flavor of food. It has been found that microorganisms are the most suitable resources for the industrial production of protease because protease-producing microorganisms are easily cultivated on a large scale. Protease yields from microorganisms are very high and different proteases produced by microorganisms have different biochemical and physical characteristics and physiological functions (Kurmar and Takagi, 1999). So the influence of selenium on yeast processing protease was analyzed after fermenting for 48 h. All operations are in triplicate and the average values are taken. As shown in Fig. 4, at the condition of non-salt, the effect of Se on the activity of alkaline protease is more obvious than that with 8% salt. Otherwise, at the condition of 8% salt and 0.5 $\mu\text{g/mL}$ sodium selenite, the alkaline protease activity of the F strain

also exhibits a slight increase, but not in the case of the JS strain. This result illustrates that selenium influences or participates in the synthetic process of yeast protease. As previously mentioned, ANOVA was repeated yielding a similar result.

It is consistent with the research of Hempel et al. (1985) that Cys-302 is the active center of alcohol dehydrogenase which fulfils a vital function relating to ADH activity. Two of the principal selenothiols (SeCys and selenohomocysteine) are α -amino acids, and can react to form peptide bonds, especially with other abundant amino acids present in yeast (e.g., glycine or glutamic acid), as reported in the paper of Bierla et al. (2012), whose detection results indicated the possible structures containing SeCys, all of which had been found in yeast by advanced MS methods.

After these effects became clear, it was necessary to evaluate the conversion rate of organic selenium to ensure that the residual inorganic selenium is safe. Residual inorganic selenite detection method follows the procedure described by Sigrist et al. (2012) with minor modifications. Briefly, after cell disruption and centrifugation, the sample solution and blank culture, respectively, flowed into a 500 μ L sample loop. The sample was then released from the loop using a directional valve by HCl carrier solution and transported to the chemifold where it was mixed with a NaBH_4 reductant in the reaction coil to produce selenium hydride (SeH_2). The reaction coil is formed by two different sections between which is located the nitrogen entrance. The liquid/vapour mixture flowed to a gas-liquid membrane separator and the gaseous hydride was transported by the nitrogen carrier stream to the quartz atomizer heated with an air-acetylene flame. The remaining liquid was removed from the separator by a peristaltic pump. An external calibration curve at Se (IV) concentration levels ranging from 2.5 to 40.0 $\mu\text{g/L}$ was used for the quantification of the Se content in the samples. Background analytical levels tested by running blank acid digestions were insignificant. The organic selenium conversion rate was calculated from the residual sodium selenite. From the results in Table 1, there is no selenium in the blank culture and the organic selenium conversion rate of the two strains both exceed 80%. In a previous study (Bierla, 2012) pointed out that the typical analytical demand from industry requires verification of the minimum content of SeMet (usually > 60%) in Se-rich yeast and a demonstration of the absence (<2%) of selenite and selenate. In this study, the organic selenium conversion rate was obtained by detecting the residual quantity of inorganic selenium.

The present study demonstrates the possible application of *Zygosaccharomyces rouxii* F strain for the selenium enrichment of sauce products, pickle and other fermenting seasonings with 0.5 $\mu\text{g/mL}$ sodium selenite, increasing its growth, and the activity of ADH and alkaline protease. Moreover, the *Saccharomyces cerevisiae* JS strain, even though it is not haloduric enough, could be used in the production process of bread, desserts, beer and wines with the same concentration of sodium selenite at the autofit addition time. In addition, more attention should be paid on the selenium-rich ability of F strain under con-

ditions of high salinity (18%), and the selenium-transfer ability of the JS strain under high sugar or alcohol conditions.

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