PRODUCTION OF SELENIUM YEAST

By

JIA-JER LIOU, YI-HUNG LIN, WEN-LING CHENG, JOHN PO-WEN YANG

and LONG-HUEI WANG

Taiwan Sugar Research Institute, Tainan, Taiwan

Abstract

A process for the production of high organo-selenium content yeast has been developed at the Taiwan Sugar Research Institute (TSRI). Selenium tolerant strains of Saccharomyces cerevisiae were obtained through traditional strain selection methods from various sources. Among the selected strains, the S. cerevisiae TSL-1 was found to give the best yield of organo-selenium when sucrose was used as the main carbon source. The organo-selenium content of S. cerevisiae TSL-1 reached about 1800 ppm after 50 hours of cultivation. A mass production process including the separation of inorganic selenium from the final product was designed and tested at a fermentation pilot plant at the TSRI.

Introduction

Selenium, a trace element that commonly exists in soil at a low level, is essential for the human body to sustain normal metabolism (Schwarz and Foltz, 1957). The discovery of its role in glutathione peroxidase (Rotruck et al., 1973) triggered the interest of many researchers on the defensive function that selenium gives against oxidative stress (Combs, 1988). Deficiency in selenium causes health problems such as metabolism disorder or, in a serious condition, Keshan disease, which is a cardiomyopathy seen in some areas where selenium is very rare in soil (Xia et al., 1994; Winnefeld, 1993; Cohen and Avisar, 1993).

Selenium has been reported as a key component of some antioxidants that can prolong life expectancy of animals and possibly human life as well (see monographs in references Burk, 1994; Prasad, 1993). Furthermore, selenium was reported to play putative roles in preventing chronic diseases, most notably carcinogenesis by some researchers (Burk, 1989; Burk, 1994; Combs, 1988). Following the recognition of the potential functions of selenium to human health, studies on the dietary intakes of selenium and related concerns, such as using selenium as a food supplement, have also prospered (Combs, 1988).

Various studies have revealed that organoselenium, mainly selenocystein and selenomethionine in proteins, gives better bioavailability of selenium in animal tests (van Ryssen et al., 1989). More importantly, it has been reported that organoselenium is safer than inorganic selenium sources as a daily nutritional supplement (Mao et al., 1991; Haas and Velten, 1993). Thus, organic sources of selenium are superior to inorganic selenium. Some yeast strains can accumulate a significant amount of organoselenium quickly when cultivated in an environment enriched with inorganic selenium (Korhola et al., 1986; Huang et al., 1988; Xie et al., 1990). Compared to selenium eggs or selenium vegetables, selenium yeast has obvious advantages in terms of the versatility of product forms. In this paper, the search for a yeast strain with high yields of organoselenium and the cultivation of selenium yeast will be discussed. A production process that can produce around 60 kilograms of selenium yeast per batch was developed using the selected strain.

Materials and methods

I. Strain

Yeast strains in the Taiwan Sugar Institute collection were used for this study, including four strains of Saccharomyces cerevisiae, strain W-3, H-1, TSL-1, and TSL-2, and one Candida utilis, strain Y-900. A commercially available bakers yeast strain acquired from a local supermarket was also used. Strains were kept in YPD agar slants that contain 2% yeast extract (Difco, Detroit, MI, USA), 1% Bacto peptone (Difco), 2% dextrose (Ishizu Seiyaku, Osaka, Japan), and 5% Bacto agar (Difco). Colonies were taken from 3-day-old YPD plate for further experiments.

II. Determination of selenium tolerance

Selenium tolerance of each strain was determined using sodium selenite (Merck, Germany) enriched YPD plates. A colony was taken from a 3-day-old YPD plate and suspended in sterilised distilled water. Proper dilutions made out of the yeast-in-water suspension were then spreaded aseptically onto selenium enriched YPD plates and also onto selenium free YPD plates for comparison. The colonies on plates were then counted after two days of incubation at 30°C to determine the colony-forming unit per mL of yeast in water suspension.

III. Cultivation method and cell mass determination

A yeast colony was taken from a 3-day-old YPD plate and aseptically transferred into a 500 mL flask containing YPD medium or YPD medium enriched with sodium selenite. Inoculated flasks were then incubated in an orbital shaker (model 706R, Hotech Instrument, Taiwan) at 30°C at 120 r/min. Cell mass was determined using dry cell weight, which was determined by taking 10 mL of suspension from the
flask; after washing once with distilled water, cells were dried in a 105°C oven, and then weighed.

IV. Quantification of organoselenium

Dried yeast samples were washed with 0.1N hydrochloric acid or distilled water for 10 minutes and then their selenium contents were determined following the fluorometric method of Alftihan (Alftihan, 1984).

V. Pilot scale production

Pilot scale production was performed at the TSRI using a 6000 L fermentor with 50% working volume. Cane sugar and urea were used as the main carbon and nitrogen sources. Produced selenium yeast was washed twice with water to reduce the concentration of selenium dissolved in liquid, presumably inorganic selenium, to be less than 1 ppm. Selenium yeast was then recovered using a spray drier that was custom made for the TSRI.

Results and discussion

In some preliminary studies on our yeast strains, we found that C. utilis was much less tolerant to selenite than all Saccharomyces strains. Among the Saccharomyces strains, the tolerance to selenite varied. Strain TSL-2 and the bakers yeast purchased from supermarket were obviously less tolerant than others. Further investigation on the tolerance to inorganic selenium was carried out on strains W-3, H-1, and TSL-1 for up to 100 ppm of selenium. Inorganic selenium in selenite form clearly inhibited the growth of strains W-3 and H-1. Approximately 25% of strain W-3 and 80% of strain H-1 cells failed to form colonies on plates containing 25 ppm selenium after two days. Furthermore, the size of single colonies on plates enriched with selenium appeared to be smaller than that without selenium. The higher the selenium content on a plate, the smaller was the size of the colonies. Among the tested strains TSL-1 showed the best selenium resistance. For selenium concentration less than 50 ppm, selenite did not affect the number of colonies forming on plates. Interestingly, the size of single colonies of TSL-1 also decreased as the selenium concentration of a plate increased. This indicated that selenite also inhibited the growth of TSL-1, but not as significantly as it did to strains W-3 or H-1.

It has been reported that when growing in a selenium enriched environment, a yeast cell incorporates selenium predominately into selenocystein and selenomethionine (Haas and Velten, 1993). However, it is difficult to distinguish chemically bound selenium from inorganic selenium absorbed to organic compounds (Abrams et al., 1990). The common practice to separate organoselenium from inorganic selenium is to use extractability of selenium into water or dilute acid (Arthur and Bechett, 1994; Khorhola and Edelmann, 1986). Although washing with water or dilute acid does not guarantee samples free of inorganic selenium physically bound to organic molecules, it is believed that inorganic selenium bound to organic molecules would be rare after washing. In the following discussion, the term “putative organoselenium” stands for the selenium content determined using the described methods, which is likely to contain both organoselenium and traces of inorganic selenium bound to organic molecules.

Batch cultures of strain TSL-1 were kept in shaken flasks to investigate the effect of inorganic selenium on cell growth based on the dry cell weight. The doubling times were 1.74, 1.86, 2.01, and 2.14 hours in cultures with initial concentrations of inorganic selenium 0, 5, 10, and 20 ppm, respectively. Cell growth in all cultures significantly slowed down 12 hours after inoculation. After 24 hours of cultivation, the cell concentration in individual cultures was 4.48, 4.16, 4.06, and 3.12 g/L corresponding to the order of increasing initial selenium concentration in the cultures. The growth rate during the exponential phase and the yield of biomass at 24 hour both show that selenite ion inhibited the growth of strain TSL-1. With 20 ppm of selenium in the form of selenite, the yield of biomass at 24 hours decreased about 30% compared to that of the selenium-free medium.

The time course of putative organoselenium content in strain TSL-1 cells cultivated in medium with various initial concentrations of inorganic selenium showed a common trend shared by the cultures enriched with selenium: the putative organoselenium content increased with time. The increase in putative organoselenium content was most profound between 8 to 12 hours after inoculation, which corresponded to the stage when cells enter into stationary phase from the exponential phase. During the stationary phase, the increase in putative organoselenium was insignificant. Since selenium is mainly incorporated into selenocystein and selenomethionine, it is expected that the putative organoselenium content could increase only when cells are actively producing amino acids. The final putative organoselenium content, which was 190, 580, and 1190 ppm for cultures enriched with 5, 10, and 20 ppm inorganic selenium, respectively, increased with the quantity of inorganic selenium initially added into the cultures.

It is not clear if yeast cells can distinguish selenium from sulfur. However, selenium seems to compete with sulfur for the positions in cystein or methionine (Haas and Velten, 1993). The results of our experiments showed that, with 20 ppm of inorganic selenium added, the rate of incorporation of selenium into organic molecules in yeast cells was roughly proportional to the concentration of selenium in the medium. It is likely that the concentration of inorganic selenium was significantly lower than sulfur containing molecules or ions in the culture medium. If the initial concentration of selenium was further increased to some certain extent, the final putative organoselenium content might increase proportionally to a level higher than 1200 ppm. However, at further raised inorganic selenium levels, the growth of yeast cells would significantly slow down. Although a medium containing a high initial concentration of inorganic selenium may yield a putative organoselenium content of more than
1200 ppm, no medium higher than 20 ppm was tested for the consideration of downstream processing.

The pilot trials of production were performed using a 6000 L fermentor with 50% working volume. Carbon source was fed with a scheduled rate that was intended to keep the yeast cells growing. Sodium selenite solution was added in after the concentration of yeast reached about 5–7% to avoid the retardation of growth at the early stage. After about 40 hours, the biomass reached the maximum of around 20 g/L and started to decrease slightly. The organic selenium content of harvested yeast was in the range of 1700 to 1800 ppm on a dry weight basis.

Conclusion

A strain of S. cerevisiae was screened and isolated from the yeast collection of the TSRI, which is able to accumulate 1150 µg putative organoselenium per gram dry cells within 24 hours. The rate of accumulation of putative organoselenium in yeast cells depends on the initial concentration of inorganic selenium added to culture media. Within 20 ppm of inorganic selenium added into culture media, the final content of putative organoselenium in selenium yeast increases with the initial level of inorganic selenium. The pilot trials using the selected strain yielded 20 g/L selenium yeast that contained up to 1800 ppm organic selenium.

REFERENCES


LA PRODUCTION DE LEVURE A FORTE TOLERANCE AU SELENIUM

JIA-JER LIOÜ, YI-HUNG LIN, WEN-LING CHENG, JOHN PO-WEN YANG et LONG-HUEI WANG

Taiwan Sugar Research Institute, Tainan, Taiwan

Résumé

Un procédé pour la production de levure à fort taux de sélénium a été développé au Taiwan Sugar Research Institute (TSRI). Des lignées de Saccharomyces cerevisiae tolérantes au sélénium furent obtenues de diverses sources par des méthodes traditionnelles de sélection. Parmi les lignées sélectionnées, le S. cerevisiae TSL-1 donna le meilleur rendement en organo-sélénium, avec le saccharose comme source principale de carbone. Le taux d’organo-sélénium du S. cerevisiae TSL-1 atteignit environ 1800 ppm après 50 heures de culture. Un procédé de production de masse, comprenant la séparation du sélénium inorganique du produit final, fut élaboré et testé dans un projet pilote au TSRI.

Mots clés: organo-sélénium, Saccharomyces.
RESUMEN
Se ha desarrollado en el Taiwan Sugar Research Institute (TSRI) un proceso para la producción de levaduras con un alto contenido de organo-selenio. Cepas de *Saccharomyces cerevisiae* selenio tolerantes fueron obtenidas mediante métodos tradicionales de selección de varias fuentes. Entre las cepas seleccionadas, la *Saccharomyces cerevisiae* TSL-1 se encontró que alcanza los mejores rendimientos de organo-selenio cuando se empleó glucosa como la principal fuente de carbono. El contenido de organo-selenio de *S. cerevisiae* TSL-1 alcanzó cerca de 1800 ppm después de 50 horas de cultivo. Se diseñó un proceso de producción en masa que incluye la separación del selenio inorgánico del producto final, el que fue comprobado en una planta piloto de fermentación en el TSRI.