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ORIGINAL PAPER

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SCREENING AND IDENTIFICATION OF YEAST ENRICHED WITH SELENIUM, ZINC AND CHROMIUM

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ABSTRACT

Background. Selenium zinc chromium is an essential nutrient for humans and animals, and the free form of selenium zinc chromium state is not easily absorbed and is highly toxic. Yeast has characteristics such as easy growth and a high absorption rate of trace elements, which make it an ideal carrier for enrichment of trace elements. Therefore, in order to solve the problems of the low absorption rate of trace elements and the difficulty of effective daily supplementation, this study will examine the use of yeast to enrich more than two kinds of nutrients at the same time and to obtain and identify the strain with a good enrichment effect, high biomass and a broad development prospect.

Methods. Sixteen yeast strains were selected in order to study their enrichment capacity for selenium, zinc and chromium, respectively, using enrichment amount and conversion rate as screening indicators. The strains were then identified by sequence analysis of the 26S rDNA D1/D2 region.

Results. The strain with a better enrichment effect of selenium, zinc and chromium was obtained as *Saccharomyces boulardii* L2. When the compound nutrients sodium selenite (30 μg/mL), zinc sulfate (200 μg/mL) and chromium chloride (100 μg/mL) were added to the medium, the selenium content of the yeast was obtained, and the zinc content and chromium content were 917.37 μg/g, 1202.3 μg/g, 680.11 μg/g, respectively. The biomass was 19.58 g/L.

Conclusion. Fortified yeast enriched with selenium, zinc and chromium was obtained using *S. boulardii* L2 as a carrier. This study provides the theoretical basis and technical support for the preparation of yeast rich in selenium, zinc and chromium.

Keywords: sodium selenite, zinc sulfate, chromium trichloride, *Saccharomyces boulardii*, screening and identification, yeast

INTRODUCTION

Microorganisms have the ability to convert trace elements in the form of inorganic salts into the form of organic matter. Trace elements in their organic form are easily absorbed and utilized by the organism.

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Yeast has become the focus of applied microbiology research at home and abroad because of its strong ability to accumulate trace elements. Yeast has the advantage of a high protein content, rich nutrition, short growth period and easy culture. In addition, it can also produce high protein biomass by using soluble sugar and organic acid (Yin et al., 2009). Under appropriate conditions, yeast cells can bind elements in ions from the environment and permanently integrate these elements into their cell structure (Kieliszek et al., 2015).

Fortified yeast is defined for the first time in "Fortified Yeast" as yeast made from *Saccharomyces cerevisiae* strains by adding carbon, nitrogen, phosphorus, vitamins and minerals in the process of fermentation and culture, and by transforming, enriching or metabolizing a certain amount of one or more nutrients produced by yeast cells within the cells, and then by separation or treatment. According to the nutrients in the product, it can be classified as chromium-enriched yeast, zinc-enriched yeast, selenium-enriched yeast, vitamin D-enriched yeast, vitamin B-enriched yeast, high protein yeast, Glutathione-enriched yeast, etc. (GB/T 35882-2018, 2018).

Suhajda et al. (2000) found that the introduction of water-soluble selenium salts into yeast media as a component of conventional batch-produced yeast media resulted in a significant uptake of selenium by the yeast. Xue et al. (2003) randomly divided 450 zinc-deficient children into three groups. The first group was supplemented with zinc-enriched yeast at the amount of 10 mg/day, the second group was supplemented with zinc sulfate at the amount of 10 mg/day and the third group was the control group. By comparing the height of the children after taking zinc, it was found that the zinc yeast group had the best effect. Therefore, the development of functional yeast rich in trace elements can prevent and treat diseases, which is of great significance to ensure the health of organisms and has broad application prospects.

Selenium, zinc and chromium are essential trace elements for the human body, which have an important impact on the normal physiological function of the body. From the perspective of biochemical metabolism and nutrition, organic selenium, zinc and chromium are better than ions in absorption and utilization, physiological and pharmacological function and safety and non-toxic side effects (Rayman, 2000). Inorganic selenium, zinc and chromium can be transformed into an organic state by yeast fermentation. The trace element-enriched yeast prepared by microbial fermentation has good biological activity, non-toxic side effects, low production cost and broad application prospect (Luo, 2011). As a kind of living microorganism beneficial to the health of the host, probiotics play a role in balancing intestinal flora in the human body, usually as a food additive. Eutrophic probiotics offer multiple benefits by integrating the advantages of probiotics and nutrients.

At present, both local and international research on the enrichment of trace elements by microorganisms mainly focuses on the transformation of single trace elements, and there are few studies on the enrichment of two or more trace elements by yeast at the same time. In this study, yeast was used to enrich two or more nutrients simultaneously. Firstly, we compared the enrichment ability of 16 yeast strains for three nutrients (selenium, zinc and chromium). In addition, strains with good enrichment and high biomass were screened and strains were identified.

MATERIALS AND METHODS

Strain activation

The 16 yeast strains used were Y1, Y2, Y3, Y4, Y5, Y6, Y7, Y8, Y9, Y10, Y11, Y12, JM, *S. boulardii* L2, *S. boulardii* L3 and *S. boulardii* YES, which were preserved in the form of freeze-dried yeast powder in the laboratory 1C419 of School of Food and Bioengineering, Shaanxi University of Science and Technology.

The lyophilized powder of 16 yeast strains stored in the laboratory was dissolved in a YPD medium and cultured in a shaking table incubator at 30°C and 180 r/min for 24 hours. After continuous activation for three generations with an inoculation amount of 5% (v/v), the number of viable cells of each strain could reach 10^8 CFU/g, which was placed in the refrigerator at 4°C for standby.

Preliminary screening of fortified yeast

Under sterile conditions, the activated strains were inoculated in YPD broth medium with an inoculation amount of 5% (v/v) and placed in the incubator at 30°C, 180 r/min for 24 hours. After the growth of the strains reached the logarithmic phase, 30 μg/mL

sodium selenite, 200 μg/mL zinc sulfate and 100 μg/mL chromium trichloride were added to the medium, respectively. Then they were placed in a 180 r/min shaker incubator and incubated at 30°C for 24 hours. The precipitation and supernatant were obtained by centrifugation at 6000 r/min at 4°C for 10 min. The contents of selenium, zinc and chromium in the supernatant and the biomass of the yeast were determined. The amount of selenium, zinc and chromium enrichment in the unit cells and their conversion rate were calculated.

Re-screening of fortified yeast

With the strains obtained from the initial screening, 30 μg/mL sodium selenite, 200 μg/mL zinc sulfate and 100 μg/mL chromium trichloride were then added into the culture medium at the same time. After comprehensive comparison, the strains with a good enrichment effect and high conversion rate were obtained, and the cell morphology was observed by a scanning electron microscope.

Conversion determination

The contents of sodium selenite, zinc sulfate and chromium trichloride were determined by inductively coupled plasma atomic emission spectrometry – ICP-AES (Zhang et al., 2020). The following method was used: Centrifuge the yeast transformation solution rich in nutrients, take 5 mL of supernatant, add nitric acid and hydrogen peroxide for digestion, dilute the nitrated sample to 20 mL with pure water, place it in ICP-AES to detect the contents of selenium, zinc and chromium, and calculate the content according to the calibration curve of corresponding standard solution established in ICP.

$$\text{Conversion, \%} = [(B - A) / B] \times 100$$

where:

A – the contents of selenium, zinc and chromium were detected,

B – total content of selenium, zinc and chromium added.

Yeast biomass determination

After shaking the flask culture, the fermentation broth was centrifuged at 6000 r/min for 10 min, the cells were collected and washed with distilled water 3 times and the yeast was collected and dried at 105°C to a constant weight to obtain the yeast biomass.

Strain identification

Morphological observations were performed first. A small amount of the strain was made into a smear, stained and observed under a microscopic oil microscope for morphological characteristics of the bacterium. The 26S rDNA D1/D2 region was then sequenced to determine the species of the strain. After the target fragment was amplified and purified, it was compared with the strains with high homology in NCBI, and the phylogenetic tree was established using software (MEGA 7.0).

DNA extraction method: according to the reference (Li et al., 2007). The principle is that under the action of the lysis solution, the protein is denatured and the DNA is freed. Since DNA is easily soluble in water and insoluble in organic solvents, and the protein surface has hydrophilic groups, it is also easy to carry out hydration and form a hydrated layer on the surface so that the protein molecules can enter smoothly into the aqueous solution to form a stable colloidal solution. In the presence of organic solutions, this colloidal stability of proteins is destroyed and denatured and precipitated. The DNA is separated by centrifugation.

PCR amplification system (50 μL). According to the reference (Suel et al., 2000). ddH_2O – 75.4 μL, 10× TaqE Buffer – 10 μL, dNTPs, 2.5 mmol/L – 8 μL, primer NL-1 – 2 μL, primer NL-4 – 2 μL, centrifuged and mixed in 2 tubes. Then add template DNA – 0.5 μL, Taq polymerase, 5 U/μL – 0.8 μL, centrifuge and mix well.

Gel preparation. According to the reference (Sipiczki et al., 2001). 1.2% agarose, 1.2 g agar plus 100 mL 1× TAE (electrophoresis buffer), 100 mL agarose plus 1–2 μL Gold View.

Electrophoresis buffer. Add 1 × TAE to the electrophoresis tank and let the liquid level be 1 mm above the gel surface.

Spotting. 6× Loading Buffer – 1 μL, DNA – 5 μL.

Electrophoresis. After spotting, set the electrophoresis conditions to 150 V for 30 min.

PCR reaction program. 94°C for 1 min, 45–55°C gradient for 1 min, 72°C for 1.5 min, 36 cycles, 4°C storage.

RESULTS AND DISCUSSION

Preliminary screening of strains

The 16 activated yeast strains were preliminarily screened and inoculated into the YPD broth at 5% (v/v). 30 μg/mL sodium selenite, 200 μg/mL zinc sulfate and 100 μg/mL chromium trichloride were added to the medium, respectively. The culture was left in a shaking table at 37°C, 180 r/min for 24 hours. The contents of sodium selenite, zinc sulfate and chromium trichloride in the supernatant and the biomass of the bacteria were measured, and the amount of selenium, zinc and chromium enriched per unit of bacteria and their conversion rates were calculated. The results are shown in Figure 1–3.

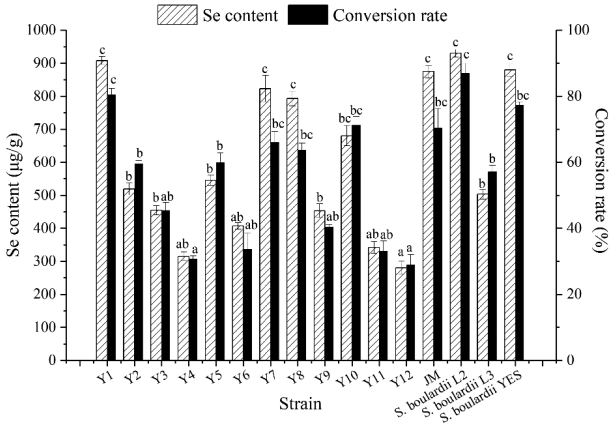

Fig. 1. Enrichment and conversion of selenium by different yeasts. Different letters indicate significant differences between groups, $p < 0.05$

It can be seen that the conversion rate of these 16 strains to sodium selenite is in the range of 20–90% from Figure 1. Among them, compared with strain Y4, the conversion rate of sodium selenite of *S. boulardii* L2 was highly significant and greater than the other strains, reaching 87%, and the selenium content of yeast cells is 930.3 μg/g. This is followed by strain Y1, whose conversion rate and selenium content were 80.46% and 908.28 μg/g, respectively.

It can be seen that among the 16 strains, the top 3 strains with a high conversion rate of zinc sulfate were strains Y1, *S. boulardii* L2 and Y11 (Fig. 2),

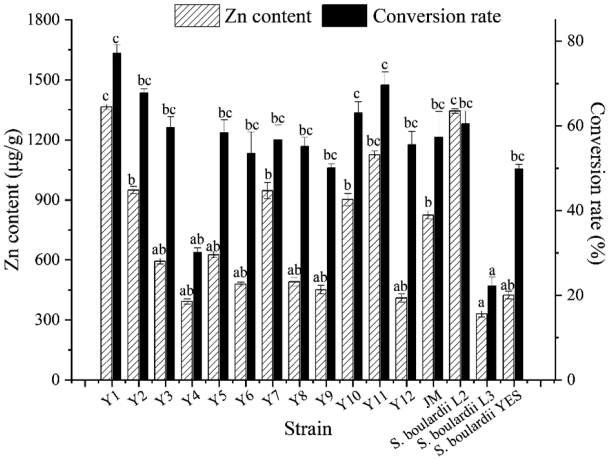

Fig. 2. Enrichment and conversion of zinc by different yeasts. Different letters indicate significant differences between groups, $p < 0.05$

which reached 77.19%, 76.19% and 68.33%, respectively, and the zinc content of the three strains were 1364.79 μg/g, 1344.05 μg/g and 1125.93 μg/g, respectively. The sodium selenite conversion rate of strain L3 was only 22.29%, and the zinc content of strain L3 was 329.69 μg/g.

It can be seen that strains Y5, Y10 and Y7 showed highly significant differences in chromium trichloride conversion compared to strain Y6, with 71.54%, 70.45% and 69.43%, respectively, and the chromium

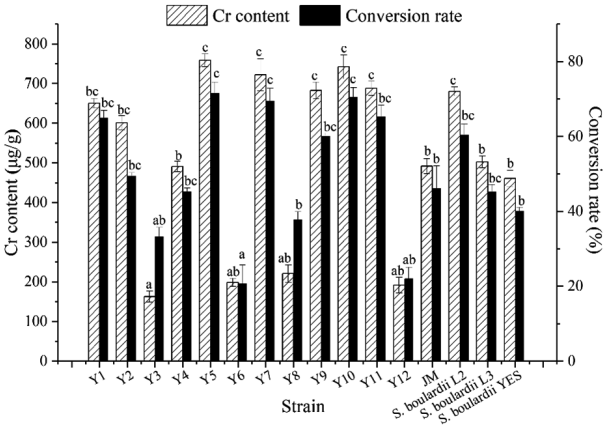

Fig. 3. Enrichment and conversion of chromium by different yeasts. Different letters indicate significant differences between groups, $p < 0.05$

contents of strains Y5, Y10 and Y7 were 758.76 μg/g, 742.49 μg/g and 722.4 μg/g, respectively (Fig. 3). The lowest chromium trichloride conversion rate of strain Y6 was 19.88%.

According to the comprehensive results of the conversion rates of sodium selenite, zinc sulfate and chromium trichloride and the nutrient content, the six dominant strains (Y1, Y5, Y7, Y10, Y11 and *S. boulardii* L2) with good single enrichment of nutrients in Figure a, b and c were screened for further screening respectively.

Re-screening of strains

Six yeast strains (Y1, Y5, Y7, Y11, Y10 and *S. boulardii* L2) obtained from the preliminary screening were re-screened. Three salt solutions were added to the culture medium at the same time (Liu et al., 2015). The culture conditions and the concentrations of sodium selenite, zinc sulfate and chromium trichloride were the same as the preliminary screening. The enrichment amount of three nutrients and cell biomass were studied. The results are shown in Figure 4–5.

It can be seen from Figure 4 that the transformation rate of inorganic selenium by the six strains screened again is L2 > Y1 > Y7 > Y10 > Y5 > Y11. The strains with high selenium and zinc content were *S. boulardii* L2, Y1 and Y7, and the selenium enrichment was more than 800 μg/g. L2, Y1 and Y7 had highly significant differences in zinc content compared to strain Y5 with 1202.3 μg/g, 1047.92 μg/g and 1044.72 μg/g, respectively. The strains with higher chromium content were Y10, L2 and Y5, which were 711.39 μg/g, 680.11 μg/g and 680 μg/g, respectively.

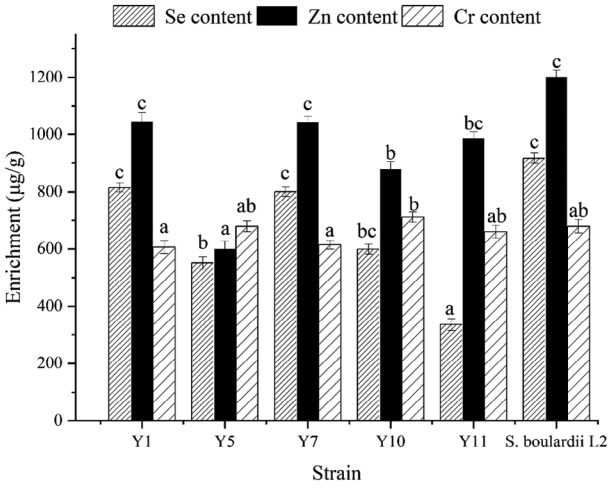

Fig. 4. Enrichment of different nutrients in 6 strains of yeast. Different letters indicate significant differences between groups, $p < 0.05$

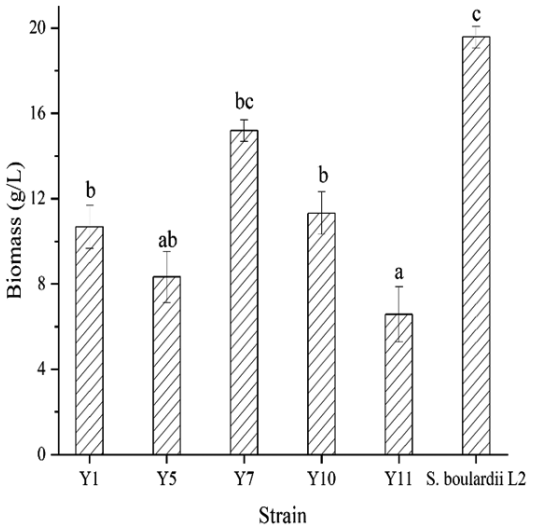

Fig. 5. Biomass of six yeast strains after screening. Different letters indicate significant differences between groups, $p < 0.05$

It can be seen from Figure 5 that the strains with high biomass are *S. boulardii* L2, Y7 and Y1, which were 19.58 g/l, 15.194 g/l and 11.323 g/l, respectively. After comprehensive comparison according to the re-screening results, the biomass of *S. boulardii* L2 and Y1 is the highest, and the enrichment of three nutrients is high. To ensure high enrichment and obtain high biomass, two strains with strong enrichment ability (*S. boulardii* L2 and Y1) were screened for further research.

Identification and phylogenetic analysis of strains

Strain identification of Y1 was carried out, and an agarose gel electrophoresis map (Fig. 6) and phylogenetic tree of the strain were obtained (Fig. 7). It can be seen from Figure 7 that the homology between strain Y1 and *S. cerevisiae* strain SX8 has reached 100%, indicating that strain Y1 is *Saccharomyces cerevisiae*. *S. boulardii* L2 is a probiotic.

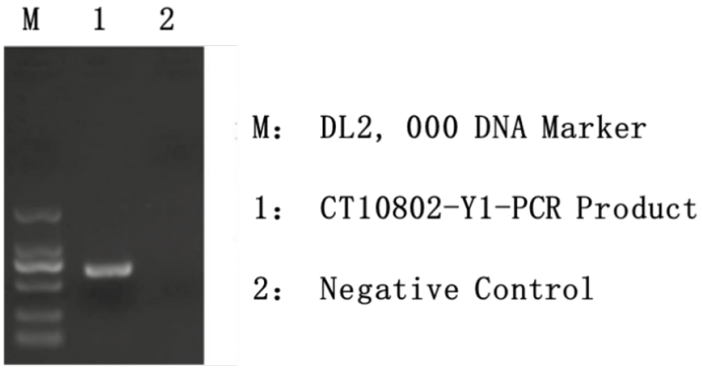

Fig. 6. Agarose gel electrophoresis of PCR products from genomic DNA from strain Y1

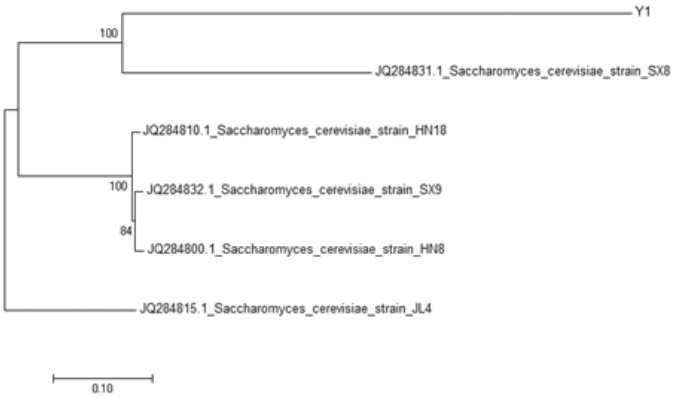

Fig. 7. Phylogenetic tree of strain Y1

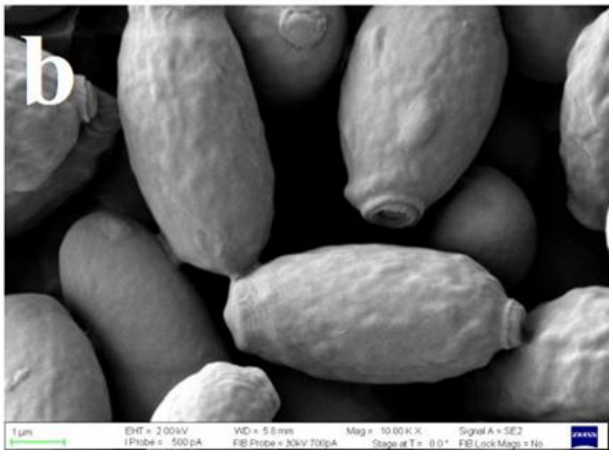

Fig. 8. Scanning electron micrograph of strain L2: a – 5 kx, b – 10 kx

Scanning electron microscope image analysis of *S. boulardii* L2

In order to observe the morphological characteristics of *S. boulardii* L2 cells and the changes on the cell surface after enrichment of nutrients, the morphology of *S. boulardii* L2 was measured by SEM. As shown in Figure 8, *S. boulardii* L2 cells were complete, ellipsoidal and large in shape and volume. In addition, a small number of cells were round, and buds appeared around some mother cells. This shows that *S. boulardii* L2 has the general characteristics of yeast: budding and reproduction. Thus, the experimental selection of salt solution concentration is feasible to verify.

DISCUSSION

Although the research on microbial enrichment of trace elements has been a popular topic at home and abroad, most of it focuses on the enrichment of single trace elements, and there are few reports on the enrichment of two or more trace elements in yeast at the same time. Therefore, it is still unclear how different yeasts enrich various trace elements and nutrients. In this study, based on the preliminary experiments, the concentration values of three salt solutions were initially selected. The enrichment of different yeasts for the same concentration, a single salt solution, was firstly derived, so that six dominant strains could be initially selected for subsequent experiments. In the re-screening, the three salt solutions were mixed at the same concentration as in the initial screening, and the medium was added to screen the strains for good

enrichment of selenium, zinc and chromium at this concentration, observe the morphology and identify the strains. The effect of single and combined salt solutions on the enrichment of the bacterium under different concentration conditions was not discussed.

In the study of selenium-rich yeast, it can be concluded that the selenium supplementing method and selenium source concentration have a great influence on selenium enrichment in yeast. The results showed that when the concentration of sodium selenite was less than 30 μg/mL, the selenium content and biomass of *S. boulardii* L2 increased with the increase of sodium selenite concentration. When the concentration reached 30 μg/mL, the selenium content and biomass reached the maximum. When the concentration of sodium selenite was higher than 30 μg/mL, the growth of the yeast is inhibited, resulting in a gradual decrease in the selenium-enriched amount and biomass of the yeast. Li et al. (2016a) observed a strain of brewer's yeast resistant to sodium selenite 5 mg/mL by scanning electron microscopy before and after selenium enrichment. The morphology of the yeast after selenium enrichment treatment differed significantly from that of the untreated yeast, with some of the cell surfaces of the organisms showing depression. This is mainly because the reduction of selenite is mainly acted upon the related reductase in the bacterium. When there is too much sodium selenite, the conversion of sodium selenite by the bacterium reaches saturation, and sodium selenite will inhibit the activity of the bacterium so that the related enzyme action activity is reduced.

In the study of zn-enriched yeast, Shariatmadari et al. (2014) added zinc salt to *Saccharomyces cerevisiae* culture medium to improve the yield of organic zinc. Therefore, the concentration of zinc sulfate in the medium has a significant effect on the zinc content and cell growth of yeast cells. This is because zinc ion is the cofactor of many biological enzymes in the organism, and with the increase of zinc sulfate concentration, it promotes the growth and development of yeast. However, the zinc concentration and osmotic pressure that yeast can tolerate are limited. When zinc salts are excessive, the enzyme activity of yeast cells is not activated and the increase of zinc ion concentration to a certain extent limits its growth, causing toxic effects on cells and inhibiting the enzyme activity of yeast cells (Guan et al., 2010).

In the study of chromium-enriched yeast, Ye Jinshao et al. (2002) studied the adsorption of heavy metal chromium by yeast and found that certain heavy metals can promote the growth of microorganisms at low concentrations, mainly due to the fact that these metals are essential components of certain enzymes in microbial cells. However, when the content of heavy metals exceeds their critical concentrations, they are toxic to microorganisms and can even kill them. Microorganisms show a certain adaptability and resistance to harmful substances and environmental changes. Chromium, as a heavy metal, has a certain toxicity to microorganisms (Liu, 2009).

From the results of this study, it can be seen that the enrichment effect of L2 on selenium, zinc and chromium in the single salt solution enrichment experiment was better than that in the combined salt solution, while the concentration was kept constant. From this, it can be tentatively inferred that the mixing of multiple salt solutions will have a common effect on the growth of the bacteria. In order to determine the mechanism of ionic interaction in salt solutions and the optimal enrichment concentration of the bacterium for different salt solutions, we should combine different salt solutions and analyze the interaction mechanism and the effect of multiple salt solutions on the growth of the bacterium through tolerance experiments under different concentration conditions. The optimum culture conditions will be determined by the corresponding surface design, which will provide a basis for the development of functional probiotic foods.

CONCLUSIONS

It was found that all 16 strains of yeast could enrich three nutrients, namely, selenium, zinc and chromium, but the amount of enrichment varied from bacterium to bacterium. The two strains with good effects on selenium, zinc and chromium enrichment were *S. boulardii* L2 and Y1, respectively. For *S. boulardii* L2, when the concentration of sodium selenite was 30 μg/mL, the selenium content and conversion rate of the bacterium were 930.3 μg/g and 87%, respectively; when the concentration of zinc sulfate was 200 μg/mL, the zinc content and conversion rate of the bacterium reached 1344.05 μg/g and 76.19%, respectively; when the concentration of chromium trichloride was 100 μg/mL, the chromium content

of the bacterium was 683.7 μg/g and the conversion rate was 58%. When 30 μg/mL sodium selenite, 200 μg/mL zinc sulfate and 100 μg/mL chromium trichloride were added simultaneously, the contents of selenium, zinc and chromium in *S. boulardii* L2 were 917.37 μg/mL, 1202.3 μg/mL and 680.11 μg/mL, respectively. The biomass was 19.58 g/L and the contents of selenium, zinc and chromium in yeast Y1 were 815.04 g/mL, 1047.92 g/mL and 606.44 g/mL, respectively. The biomass was 11.323 g/L. Strain Y1 was identified as *Saccharomyces cerevisiae*. By scanning electron microscopy (SEM) observation, the *S. boulardii* L2 were intact and ellipsoidal with a large morphological volume. The study provides a theoretical reference and technical support for the preparation of selenium, zinc and chromium fortified yeast, which in turn leads to the development of functional probiotic foods, allowing the intake of adequate amounts of trace elements while supplementing probiotics, which can be considered a double benefit.

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