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Selection of a high-biomass, chromium-rich yeast strain and optimization of cultivation conditions

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Saccharomyces cerevisiae **LZ-53 was selected from 240 primary yeast strains from different genera and species, whose chromium (Cr) resistance and biomass were higher than others were. The highest biomass and Cr content of the strain was obtained in 30 h at 28°C and 200 rpm, when 20 ml of the culture in 250-ml shake flasks was grown in wort containing 1200 μg/ml Cr. The initial pH was adjusted to 6.0. The optimal inoculum volume was 10% (v/v). The Cr content of the cells was determined by neutron activation analysis. Under the optimized cultivation conditions, the Cr content reached 3248 μg/g.** *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 195–198.

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Introduction

Chromium (Cr), both trivalent inorganic and organic forms, has been proposed to act as an initiator of insulin action in animals and human beings. Cr deficiency induces symptoms resembling diabetes, such as glucose intolerance impairment resulting in an increasing requirement for insulin, and Cr supplement can alleviate the symptoms [1–3,16,17,19]. The response to Cr in an organism is dependent not only upon the amount of supplemental Cr, but also on its chemical species. Absorption and bioavailability of inorganic Cr were lower than those of organic Cr compounds in humans and animals [6,7,12–14,22].

Yeast has a certain enrichment ability for trace elements. It can convert inorganic Cr to organic species and can be used as a Cr carrier. Thus, its bioavailability in the human body is improved, which can reduce the levels of blood sugar, blood fat, and cholesterol in diabetics [4,14,16]. However, high concentrations of Cr in the culture medium will restrain the growth of yeast and result in low productivity. The selection of a high-biomass, Cr-rich yeast strain is critical to the application of Cr-rich yeast. In addition, suitable cultivation conditions also have a great influence on yield. Most current studies have focused on the chemical characteristics of Cr and the Cr-combined forms in it [9,10,12,14,16], but a screening study of high-biomass, Cr-rich yeast is still not available. The present paper is mainly aimed at the screening of high-biomass, Cr-rich yeast strains and optimization of cultivation conditions. Neutron activation analysis (NAA) affords high sensitivity, good accuracy, and nondestructiveness, and no matrix effect and was adapted to determine the Cr content in Cr-rich yeast [5,8,10,12,13].

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Materials and methods

Chemicals

Chromium acetate, yeast extract, peptone, glucose, sucrose, and maltose were of analytical grade and purchased from the Sino-American Biotechnology (Luoyang, Henan Province). Wort was obtained from the Tsingdao Brewery (Tsingdao, Shandong Province). Molasses were obtained from the Gannan Pharmaceutical factory, Jiangxi Province.

Yeast strains

Two hundred and forty primary yeast strains from different genera and species, offered by the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China), were tested to identify a strain with high biomass and high enrichment of Cr. The yeast strains were maintained on YEPD agar slants and stored at 4°C.

Medium and culture conditions

YEPD medium contained (per liter): 10 g yeast extract, 10 g peptone, and 20 g glucose; for slants, 12 g agar was added. YEPS medium contained (per liter): 10 g yeast extract, 10 g peptone, and 20 g sucrose. YEPM medium contained (per liter): 10 g yeast extract, 10 g peptone, and 20 g maltose. Wort medium contained 10°Brix total sugar. Molasses medium contained (per liter): 40 g molasses containing 48% sucrose, 0.5 g $(NH_4)_2SO_4$, and 1 ml H_3PO_4. The pH of the media was 6.0 [15,20]. Concentrations of Cr in media indicated concentrations of Cr ion, not that of chromium acetate. Seed cultures were grown in a 125-ml Erlenmeyer flask containing 30 ml YEPD liquid medium at 28°C on a rotary shaker (200 rpm) for 20 h. Fermentation tests were conducted at 28°C on a rotary shaker (200 rpm) for 24 h.

Selection of yeast strain

Primary screening: Cells of strains for selection were suspended in 1 ml sterile water and starved for 4 h at room temperature. The suspension of starved cells was inoculated on

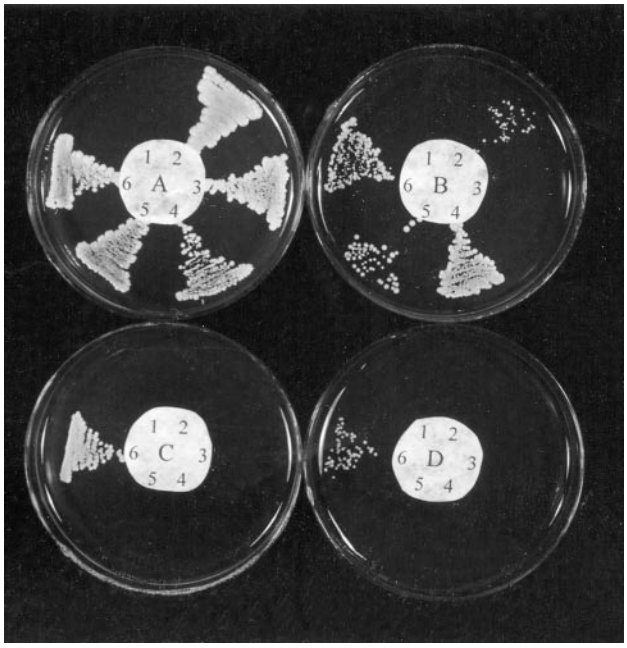

Figure 1 Growth of different strains on plates with different Cr concentrations: (A) 400, (B) 800, (C) 1200, and (D) 1600 μg/ml. (1) *Candida maltosa* LZ-4; (2) *Saccharomyces cerevisiae* LZ-207; (3) *C. krusei* LZ-27; (4) *Sporobolomyces roseus* LZ-235; (5) *Schizosaccharomyces pombe* LZ-215; (6) *S. cerevisiae* LZ-53.

YEPD plates containing Cr at different concentrations and incubated at 28°C for 3 days. Strains that grow on plates containing higher concentration of Cr were selected.

Replica screening: Each primary selected strain was inoculated in a 125-ml Erlenmeyer flask containing 30 ml YEPD liquid medium and incubated at 28°C on a rotary shaker (200 rpm) for 20 h as seed culture; 5 ml seed culture was inoculated in a 250-ml Erlenmeyer flask with 50 ml YEPD liquid medium containing Cr and incubated at 28°C on a rotary shaker (200 rpm) for 24 h. Cells were harvested to determine the biomass and the Cr content of the cells.

Determination of the biomass and cell dry weight

The fermentation cultures were centrifuged for 5 min at 4000 rpm. Cells were washed once with distilled water and centrifuged as above. Biomass was determined as the milligrams of wet cell weight per 100 ml culture (mg/100 ml). For the determination of dry cell weight, 1 g of wet cells was weighed and then air-dried for 6–8 h at 80°C to constant weight; 1 g of wet cells yielded about 0.23 g of dry cells in our test conditions.

Determination of Cr content of cells

Yeast cells were gathered by centrifugation for 5 min at 4000 rpm, and washed with distilled water three times. Then, the cells were air-dried 6–8 h at 80°C to constant weight and ground using a mortar and pestle. The Cr content of the cells was determined by NAA [10]. Samples of the disrupted cells were irradiated in a Heavy Water Reactor at the Chinese Institute of Atomic Energy (Beijing, China) at a neutron flux of 5.0×10^{13} n cm^{-2} s^{-1} for 4 h. The radioactivity induced in the samples was counted by a high-purity Ge detector after 12 days of decay. The certified reference materials, Tomato Leaves (EPS-1; Institute of Environmental Chemistry, Academia Sinica, Beijing, China) and Poplar Leaves (GBW-07604; State Technical Supervision Bureau, China), were used to check the accuracy of the analytical method.

Calculation of total Cr content

Total Cr content was calculated using the formula: total Cr content (μg/100 ml)=biomass (g/100 ml)×Cr content (μg/g dry cells)×0.23, where 0.23 means that 1 g of wet cells yields about 0.23 g of dry cells.

Statistical analysis

All fermentation cultures were run in triplicate. Data were analyzed statistically using Data Analysis and Technical Graphics, origin 5.0 (Microcal Software Inc., Seattle, WA).

Results

Screening of Cr-resistant strains

Fifteen strains of yeast that exhibited comparatively high Cr resistance were selected out of 240 strains, according to their growth on YEPD plates containing Cr at different concentrations. Six of the strains were inoculated on four plates with different Cr concentrations: 400, 800, 1200, and 1600 μg/ml. The results are shown in Figure 1. Strain LZ-53 had the highest Cr resistance.

Then, 15 strains of yeast that exhibited comparatively high Cr resistance were cultured in YEPD media containing 500 and 1000 μg/ml Cr. The biomass of these strains was determined. The biomass of strain LZ-53 was the highest. According to the biomass and Cr resistance property of yeast strains, strain LZ-53 was used as the initial strain for optimization of the cultivation conditions.

Optimization of cultivation conditions for strain LZ-53

Effect of the media: Strain LZ-53 was cultivated in YEPD, YEPS, YEPM, wort, and molasses media containing 1000 μg/ml Cr. The biomass and Cr content of the cells and the total Cr content of strain LZ-53 in different media containing 1000 μg/ml Cr are shown in Table 1.

Biomass of strain LZ-53 grown in different media was greatly different. Strain LZ-53 had higher biomass in wort, but the Cr content of strain LZ-53 had almost no change in different media. The total Cr contents of strain LZ-53 in wort and YEPD medium were higher than in other media. Considering the biomass and the total Cr content, the optimal medium for cultivation of strain LZ-53 was wort.

Table 1 Comparison of the biomass and Cr content of cells and the total Cr content of strain LZ-53 in media containing 1000 μg/ml Cr

Culture medium	YEPD	YEPS	YEPM	Wort	Molasses
Biomass (mg/100 ml)	2220±48	1860±51	1980±35	2400±47	1770±46
Cr (μg/g dry cells)	1994±42	1998±38	2026±43	2090±52	2045±39
Total Cr (μg/100 ml)	1018±36	855±44	923±38	1154±51	833±50

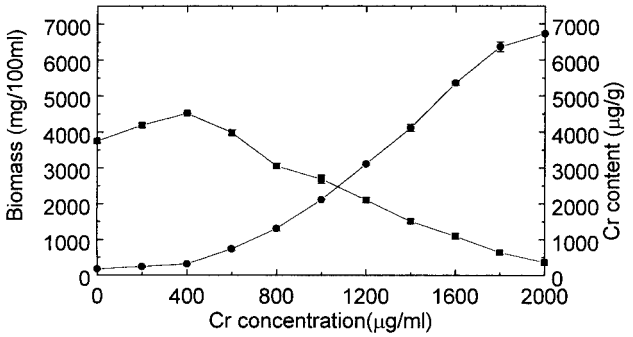

Figure 2 Effect of the Cr concentration of media on the biomass and Cr content of strain LZ-53. Biomass (■), Cr content (●). Values are means of three different cultures. Bars represent SD.

Effect of sugar concentration: Wort with different total sugar concentrations and 1000 μg/ml Cr was used to determine the effect of sugar concentration on strain LZ-53. The biomass and Cr content of the harvested yeast cells were determined. The results indicated that the total sugar concentration influenced the biomass of strain LZ-53, but had little effect on the Cr content of the cells. Therefore, the sugar concentration of wort for cultivation of strain LZ-53 was 10°Brix.

Effect of Cr concentration: Strain LZ-53 was cultivated in 10°Brix wort with different concentrations of Cr. The biomass and Cr content of harvested yeast cells were determined. The results (Figure 2) indicated that the biomass was low when the Cr content in the cells was high, and that the Cr content was low when the biomass was high. A suitable Cr concentration in media was selected to be 1200 μg/ml, which made the total Cr enrichment amount maximum.

Effect of initial pH: Wort containing 10°Brix total sugars and 1200 μg/ml Cr was adjusted to pH levels ranging from 4.0 to 8.0. The effect of pH on the biomass and Cr content of strain LZ-53 was determined. The maximum biomass of strain LZ-53 was obtained at initial pH 6.0, whereas the maximum Cr content of the strain was observed at initial pH 4.0. With the increase of pH, the Cr content of the cells decreased (Figure 3). Considering the total Cr enrichment of strain LZ-53, the initial pH of the medium was adjusted to 6.0.

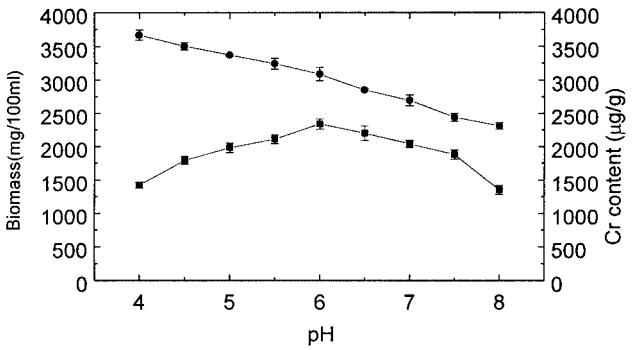

Figure 3 Effect of pH of the medium on the biomass and Cr content of strain LZ-53. Biomass (■), Cr content (●). Values are means of three different cultures. Bars represent SD.

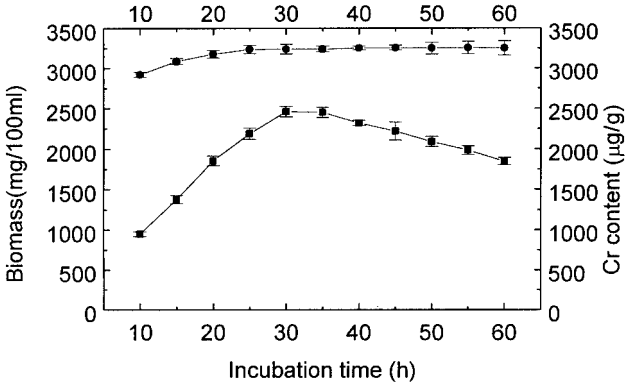

Figure 4 Effect of incubation time on the biomass and Cr content of strain LZ-53. Biomass (■), Cr content (●). Values are means of three different cultures. Bars represent SD.

Effect of the volume of medium: A different volume of wort with 1200 μg/ml Cr concentration and pH 6.0 was added into each 250-ml flask. After incubation at 28°C on a rotary shaker (200 rpm) for 30 h, samples were taken to determine the biomass and Cr content of the cells. The volume of the medium significantly affected the biomass and Cr content of the cells. Both biomass and Cr content descended with the increasing volume. When the volume of the medium was 20 ml, the biomass and Cr content of strain LZ-53 were the highest.

Effect of inoculum volume: Strain LZ-53 was cultivated in a 250-ml Erlenmeyer flask containing 20 ml wort with 1200 μg/ml Cr, pH 6.0, at 28°C on a rotary shaker (200 rpm) for 30 h. Samples were taken to determine the biomass and Cr content of the cells. The inoculum volume had little effect on the biomass and Cr content. Therefore, the inoculum volume for cultivation of Cr-rich cells of strain LZ-53 was selected to be 10%.

Effect of incubation time: The biomass and Cr content of strain LZ-53 were determined at different incubation times. The highest biomass was achieved at 30 h, without significant change of the Cr content between 25 and 60 h (Figure 4). Therefore, the incubation time was recommended to be 30 h.

Comparison of biomass and Cr content under initial and optimal conditions: Strain LZ-53 was cultivated for 30 h in 10°Brix wort with 1200 μg/ml Cr, pH 6.0, 20 ml fermentation medium in a 250-ml flask and 10% inoculum volume. The biomass and Cr content in the harvested yeast are listed in Table 2.

Table 2 Cr enrichment of strain LZ-53 under optimal conditions

Parameter	Culture condition	
	Initial	Optimal
Biomass (g/100 ml)	2420±75	2340±71
Cr (μg/g)	2085±63	3248±65
Total Cr (μg/100 ml)	1161±68	1748±70

Discussion

Trivalent Cr has been proposed to act as an initiator of insulin action in animals and human beings. Cr exists in different materials. Brewer's yeast, as carrier of trace elements, was used in the treatment of diabetes more than a century ago. Later studies showed that the organic Cr in brewer's yeast is beneficial to the treatment of diabetes [11,18]. Szalay [21] reported a Cr-rich yeast strain with Cr content of 2000 μg/g. Demirci and Pometto [9] studied the effect of fermentation mode on the Cr content of yeast and their result was 2966 μg/g. In this study, *S. cerevisiae* LZ-53 was selected from 240 primary yeast strains from different genera and species whose Cr resistance and biomass were higher than others. The Cr content of yeast increased with resistance of the strain to Cr.

The Cr concentration, pH, and volume of the medium affected the biomass and Cr content of the Cr-rich yeast. With a high Cr concentration in the medium, the biomass was low and the Cr content in the cells was high. On the contrary, the Cr content in the cells was low when the biomass was high. The largest biomass did not occur in the medium with the lowest Cr concentration, but a Cr concentration of 400 μg/ml. And there was a trend that the biomass increased slightly with increasing Cr concentration at less than 400 μg/ml. A possible reason was that low Cr concentration might stimulate growth. But the mechanism remains for further study. An acidic medium was helpful to Cr enrichment, but harmful to growth of the yeast. Poor aeration inhibited growth and Cr enrichment.

The Cr enrichment ability of strain LZ-53 has been greatly improved after the optimization of its cultivation conditions. Under optimal cultivation conditions, the Cr content of the yeast reached 3248 μg/g in contrast to 2085 μg/g under the initial cultivation conditions.

Absorption and bioavailability are dependent on the species of Cr in yeast cells. Trivalent Cr leads to improved blood glucose, insulin, and lipid variables, while Cr (VI) is harmful to organisms [19]. The absorption of inorganic Cr compounds was lower than that of organic Cr compounds in humans and animals with Cr (III) supplementation [3,7,14]. In this study, although a yeast strain with high Cr enrichment has been obtained, the species of Cr in yeast cell is still unclear. Therefore, further study on the species of Cr in yeast cells and their biological activities is needed.

Acknowledgements

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