Isolation and Some Characteristics of Cadmium-resistant Yeast[†]

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We have obtained cadmium-resistant yeast strains by repeated training the parent strain with medium containing a definite concentration of cadmium. A metal, such as cadmium has a toxic effect on yeast¹⁾ and is one of the mutagens.²⁾ Therefore, the cadmium-resistant strain is assumed to arise through mutation and selection by cadmium.³⁾ Moreover, we have obtained another type of cadmium-resistant yeast strain by ultraviolet ray irradiation. Thus, we are keeping four kinds of cadmium-resistant yeast strains. We will report here some characteristics of those newly isolated cadmium-resistant yeast strains.

Two auxotrophic heterothallic haploid strains of *Saccharomyces cerevisiae* 101* (*a ade7 ura1*) and 102* (*a, ade6, ura4, his4, trp1, leu2, lys2, tyr1, arg4*) were used as the parent sensitive strains. The diploid strain 111 obtained by mating 101 and 102 was also used.

The parent sensitive strain (101, 102 or 111) was inoculated in liquid MH medium (cane sugar 100 g, peptone 5 g, KH_2PO_4 5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1 g, malt extract [Bé 8°] 360 ml and deionized water 1000 ml) containing 0.1 mM cadmium sulfate,** and cultured under shaking conditions at 30°C. The culture reached to a stationary phase after 72 hr via a long lag phase. Cells grown were transferred into a new liquid MH medium containing 0.2 mM cadmium sulfate. After growth, the cells were transferred into new liquid MH medium containing a higher concentration of cadmium. By these serial transfer, we obtained a resistant strain which grew in the presence of 0.5 mM cadmium sulfate. However, the substrain could not grow in a concentration of cadmium sulfate higher than 0.6 mM. Cells cultured in liquid MH medium containing 0.5 mM cadmium sulfate were spread on MH agar medium containing 0.5 mM cadmium sulfate. After 48 hr incubation at 30°C, one of the colonies on the plate was chosen as a resistant strain and kept on a MH agar slant

containing 0.5 mM cadmium sulfate. The cadmium-resistant strains obtained were named 301, 302 and 331 derived from strain 101, 102 and 111, respectively.

Procedures to get the cadmium-resistant mutants by ultraviolet treatment were as follows. The yeast cells of sensitive strain 101 grown in liquid MH medium were washed with deionized water and resuspended in phosphate buffer (0.05 M) at a concentration of 10^7 cells/ml. A 1 ml portion of the cell suspension in an open petri dish was irradiated. The source of the ultraviolet ray was a Manasuru lamp having almost monochromatic radiation at 235.7 nm. Irradiation performed for 3 min from a distance of 20 cm in a dark room. The cell suspension was kept for 1 hr in a dark room after irradiation to avoid photoreactivation. The cadmium-resistant mutant in the cell suspension was selected by the replica method. After ultraviolet irradiation, the highest concentration of cadmium in which the resistant strain grew was 0.2 mM. One colony grown on a plate containing 0.2 mM cadmium sulfate was isolated as a resistant strain and named 30EU.

To estimate the degree of cadmium resistance of these strains, the cadmium gradient agar plate method³⁻⁵⁾ was used. A rectangular plastic dish (10 × 16 cm) with a linear cadmium gradient agar plate containing 3 mM cadmium sulfate on one side and no cadmium on the opposite side was prepared. The strains were pre-cultured in liquid MH medium without cadmium for 48 hr before assaying their resistance. The cell suspension suitably diluted (*ca.* 10^4 cells/ml) was streaked on the cadmium gradient agar plate using a painting brush, and the growth range on the plate was measured after 72 hr incubation at 30°C.

As shown in Fig. 1, resistant strains 301, 302 and 331 grew in the higher cadmium concentration region compared with the parent strains 101, 102 and 111. Although 30EU showed a lower degree of resistance than any other resistant strain, it had significant resistance compared with its parent strain 101. Each resistant strain kept the same level of cadmium resistance after five transfers onto the cadmium free slant. This result indicates that strains 301, 302, 333 and 30EU were stable cadmium-resistant strains.

The three haploid resistant strains 301, 302 and 30EU showed no change in auxotrophic characteristics. However, 302 derived from 102 showed respiratory deficiency in examinations by the TTC (triphenyl tetrazolium chloride) over layer method⁶⁾ and in glycerol selective medium. This result agrees with the Lindgren's result⁷⁾ that cadmium is a mutagen for respiratory deficient mutation of yeast. However, the other resistant strains, 301, 331 and 30EU did not show any respiratory deficiency characteristics.

The uptake of cadmium by the resistant strains has been studied. Cells cultured in liquid MH medium containing a definite concentration of cadmium for 48 hr to stationary phase were collected, washed twice

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** Cadmium concentration was determined using an atomic absorption spectrophotometer.

FIG. 1. The Degree of Cadmium Resistance in Various Yeast Strains.

The cell suspension of sensitive strain (101, 102, 111) and resistant strain (301, 302, 331, 30EU) was streaked on a linear cadmium gradient agar plate containing 3 mm of cadmium sulfate on right side and no cadmium on left side of the dish.

TABLE I. THE GROWTH YIELD AND CADMIUM CONTENT IN CELLS IN CADMIUM-RESISTANT STRAINS

Strain	Cadmium content in medium(mm)	Growth yield (mg dry wt./ml culture)	Cadmium content in yeast (mg/g)
301	0.5	2.8	1.99
302	0.5	3.8	3.15
331	0.5	2.7	1.76
30EU	0.2	1.7	1.36

with deionized water and dried at 105°C for 48 hr. Wet combustion of dried cells was achieved in solution ($\text{HNO}_3:\text{HClO}_4=10:1$) using H_2O_2 as an oxidant at 100°C, and the treated solution was diluted to a definite volume with deionized water for cadmium analysis. The amount of cadmium was determined by a Hitachi 207 atomic absorption spectrophotometer at 228.8 nm. As shown in Table I, strain 302 shows a better growth yield and a higher cadmium content than 301 and 331, however, 30EU shows a lower growth yield and lower cadmium content. One of the mechanisms of cadmium resistance in some bacteria might be impermeability to cadmium. For example, the uptake of cadmium by cadmium-resistant *Staphylococcus* was less than that of a sensitive strain.⁸⁾ However, in our case, a fair amount

of cadmium accumulated in cadmium-resistant yeast cells. Our isolated cadmium-resistant strain of yeast is unique and that this strain might be used to remove cadmium from industrial waste water containing cadmium.

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