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Cultural Conditions of Heavy Metal-ionTolerant Microorganism andAccumulation of Heavy Metal-ion into the Cells.

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重金屬耐性菌株의 培養條件 및 菌體內 蓄積

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ABSTRACT

The cultural conditions and the intracellular accumulation of cadium was studied using a cadmium tolerant yast strain B-7 which had been isolated from activated sludge collected from a zinc mining area.

The organism was able to grow in a medium containing 3,000 μg/m*l* of cadmium-ion. (Cd^{++}) Optimum conditions for the growth of the organisms were 20~22°C and pH 5.0~8.0 under aerobic condition. The maximum cadmium accumulation was observerd when the organism was grown at pH 6.0. The growth of B-7 was not affected by the addition of a silicone-based antifoamer, which stimulated the intracellular accumulation of cadmium. The intracellular cadmium accumulation started after the cell ceased to grow. One gram of cells accumulated 34.17mg of cadmium when the organism was grown in a medium containing 500μg/m*l* of cadmium and 0.2%, v/v silicone-based antifoamer at 28°C for 48 hours with shaking. About 73% of the accumulated heavy metal by the organism was found in the cytoplasm.

INTRODUCTION

Cadmium and zinc are refined from the zinc ore and have been used in many industries such as metals plating, cosmetics and so on. The increased use of cadmium raises the concerns of environmental pollution by the heavy metal.

Some authors (1—4) studied the Cd^{++} tolerant bacteria. In earlier study (4) high Cd^{++} tolerant microorganisms, C-7 and B-7 were isolated from a zinc mining districts where cadmium pollution was found very often. C-7 was identified as *Erwinia* sp., a cadmium-ion tolerant bacterium, and the other B-7 was cadmium-ion tolerant yeast. The strain C-7 grew in a medium containing 2,800μg/m*l* of Cd^{++} and the maximum intracellular accumulation of cadmium was measured to be 28.60 mg/g dried cells during cultivation in a medium

* Studies on the Cadmium-ion Tolerant Microorganisms, part III. Part II is in the References; 5.

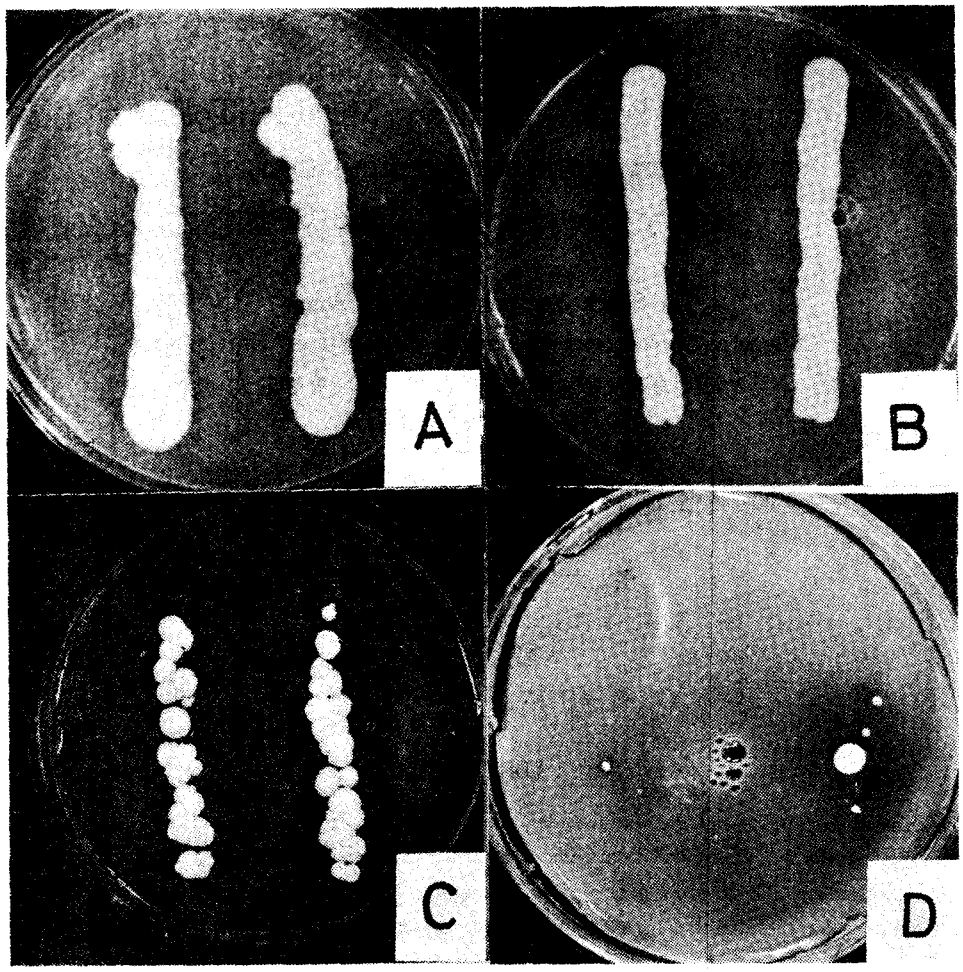

Fig. 1. Growth of Cadmium-ion Tolerant Yeast, B-7, in Different Cadmium-ion Concentration.

Dilution method in solid media was used for determining the degree of cadmium tolerance.

A ; the strain was cultivated at 28°C for 3 days in a medium without cadmium ion.

B ; the strain was cultivated at 28°C for 3 days in a medium containing 1,000μg/m*l* of cadmium ion.

C ; the strain was cultivated at 28°C for 5 days in a medium containing 2,000μg/m*l* of cadmium ion.

D ; the strain was cultivated at 28°C for 7 days in a medium containing 3,000μg/m*l* of cadium ion.

containing 50μg/m*l* of Cd^{++} under aerobic condition at 28°C for 24 hours (4), and its growth was strongly inhibited by mercury but not by other heavy metal ions (5). Various species of bacteria have been reported to be able to grow whilst little is known about the cadmium tolerance by yeast.

Heldwein (7) was observed that Cd^{++} was about 100 times more toxic than cobalt to *Saccharomyces cerevisiae* and that growth was inhibited immediately after the addition of cadmium into the culture.

otherwise cadmium-ion tolerant yeast have been

rarely studied. This paper deals with some microbiological characteristics and the intracellular accumulation of cadmium by a Cd^{++} tolerant yeast strain B-7.

MATERIALS AND METHODS

Cadmium-ion tolerant microorganism

The Cd^{++} tolerant yeast strain, B-7 was used in this study. The organism had been isolated from activated sludge collected from a zinc mining area, Sanmak Mining Company, Kalsan-ri, Kyung Sang Pook Do, Korea (4).

Cultvation

The strain was cultivated in basal medium (20g of glucose, 15g of peptone, 5g of yeast extract, 1g of NaCl, 300mg of $MgSO_4 \cdot 7H_2O$, 100mg of KH_2PO_4 and 1*l* of distilled deionized water) at pH 6.0.

The cells were gtown in a 500m*l* shaking flask containing 100m*l* of the basal medium on a reciprocal shaker (110~120 strokes/min.) at 28°C for 24 hours. The cells were harvested by centrifugation, and were washed with 0.9% NaCl solution twice. The growth was determined by measuring the optical density of the culture at 660nm and dried cell weight (g).

Determination of cadmium-ion tolerance

The tolerance against Cd^{++} was determined dilution method using solidified basal medium supplemented with various concentration of Cd^{++} as cadmium nitrate (Hyashi Pure Chemical Industuies Co. Ltd., Japan), as described previously (4).

Analysis of cadmium

The accumulated cadmium content in the cells was determined by atomic absoorption method using Unicam (model SP-1900) atomic absorption spectrophotometer with 2,288Å wave length as described previously (4).

Fractionation of tolerant yeast cell components

The harvested cells were resuspended in water and disrupted by a ultrasonic oscillator (60Hz, 30 min.) before centrifuged at 4,000 xg for 10 min to separate the cytoplasm fraction from homogenate of the cell. The cytoplasm fraction was further centrifuged at 16,000 xg for 20 min in order to separate precipitate and supernatant fraction from the cytoplasm.

RESULTS

Cadmium tolerance of the strain B-7

The strain B-7 is a high Cd^{++} tolerant yeast which was isolated at the above described area. The strain showed good growth in aqueous medium and on agar plate medium containing 2,000μg/m*l* of Cd^{++} By the dilution method using solid medium Cd^{++} tolerance of the strain was determined as 3,000μg /m*l* (Fig. 1).

Optimal temperature and pH for growth

In general, the strain B-7 grew very well between 20 to 22° C and the range of pH 5.0 to 8.0 The optimal growth pH was indicated near 6.0 as shown in Fig. 2.

Effect of shaking for growth

The growth of the strain B-7 was increased up to about 20% by shaking compared with static cultivation without Cd^{++} No effects on growth were observed in the presence of Cd^{++} up to 100μg/m*l*.

Effect of pH on accumulation of cadmium

In order to examine the effect of pH on intracellular accumulation of cadmium, the strain was cultivated in the medium containing 100μg/m*l* of cadmium at various initial pH from 4.0 to 8.0.

For the accumulation of cadmium, optimal pH was 6.0, and the accumulation was sharply decreased at other pH ranges, as shown in Fig. 2.

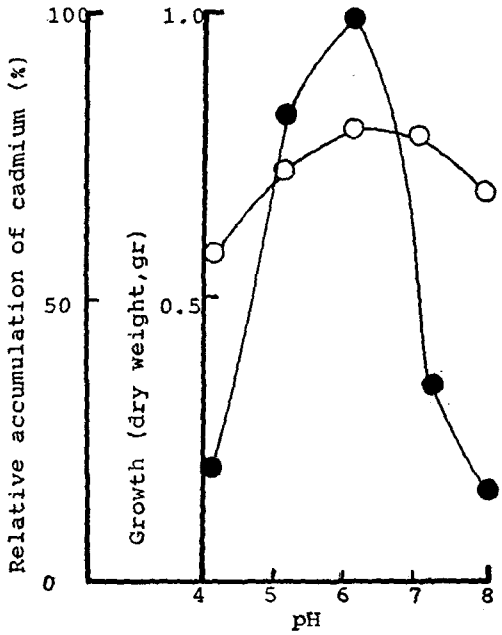

Fig. 2. The Effect of pH on Accumulation of Cadmium.

Cultivation was carried out on a reciprocal shaker at 28°C for 48 hours.

—●— relative accumulation of cadmium ;

—○— cell growth.

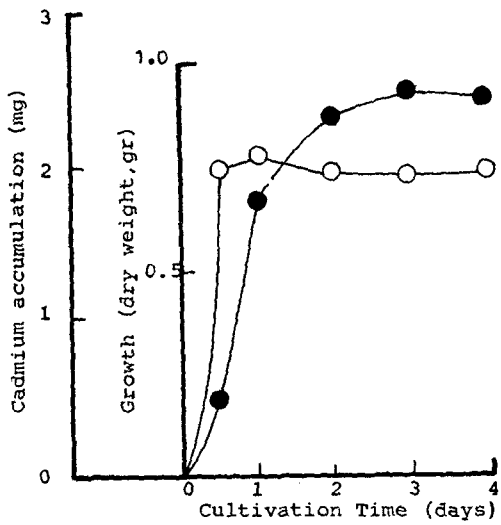

Fig. Time Course of Accumulation of Cadmium and Cell Growth.

Cultivation is described in Fig. 2.

—●— cadmium accumulation ;

—○— cell growth.

Time course on accumulation of cadmium by the cells

Fig. 3 shows the time course of cell growth and intracellular accumulation of cadmium in the medium containing 100μg/ml of cadmium. The maximum cell growth was obtained after the cultivation of 12 hours and thereafter maintained the same population of cells. But the intracellular accumulation of cadmium was abruptly increased during the stationary phase and then reached maximum at 48 hours after the inoculation.

Accumulation of cadmium into the cell

The strain was cultivated at 28°C for 48 hours on a reciprocal shaker in medium containing 100 μg/ml, respectively, and determined intracellular cadmium contents.

The results are summarized in Table 1. The intracellular accumulation of cadmium was measured to be 21.67mg/g, 34.17mg/g, and 26.98mg /g of dried cells during cultivation in each medium containing 100μg/ml, 500μg/ml, and 1,000μg/ml of cadmium under aerobic condition, respectively.

Effect of antifoamer on the accumulation of cadmium

In order to determine the effects of antifoamer, the organism was cultivated in the medium containing 100μg/ml of cadmium and 0.2% of silicone KM-70 (Shin-Etsu Chemical Industry Co. Ltd., Tokyo, Japan) as an antifoamer, and assayed intracelular cadmium.

As shown in Table 2, no effects were observed on its growth with antifoamer, whereas intracellular accumulation of cadmium was effectively increased up to 46.5% as compared with the absence of antifoamer.

Intracellular distribution of cadmium

Intracellular distribution of cadmium in the Cd^{++} tolerant yeast cell was examined.

The cells which was cultivated at 28°C for 48 hours in the medium containing 100μg/ml of cadm-

Table 1. Accumulation Cadmium into Cadmium-ion Tolerant Yeast, B-7, Cells.

Added cadmium conc. (ug/ml)	Accumulated cadmium content (mg/g dried cells)	Recovery (%)
100	21.67	38.00
1,500	34.17	38.90
1,000	26.98	20.00

Table 2. The Effect of Silicone on Growth and Accumulation of Cadmium.

	Growth (g, dried cells)	Relative (%)	Accumulated Cadmium content (mg/g dried cells)	Relative (%)
Control	0.8103	100	12,969	100
Silicone (0.02%)	0.8214	100.4	19,007	146.5

Table 3. Distribution of Cadmium in the Cell Fractions.

Fraction	Accumulated cadmium (mg)	Relative (%)
Precipitate of cell homogenate obtained by centrifugation at 4,000xg for 10 min.	10.34	27.41
Precipitate of cell homogenate obtained by centrifugation at 16,000xg for 20 min.	21.63	57.34
Supernatant of cell homogenate obtained by centrifugation at 16,000xg for 20min	5.75	15.25

The cadmium-ion tolerant yeast, B-7, was cultivated at 28°C for 48 hours in the basal medium containing 100μg/ml of cadmium ion and 0.2% of silicone. The factionation was carried out as described in Material and Methods.

ium ion and 0.0% of silicone, was harvested by centrifugation at 5,000xg for 10 min and washed with 0.9% NaCl solution twice. The yeast cells were disrupted with a sonic oscillatorto obtain cell homogenate. In order to check the distribution of cadmium accumulated in the cells, the fractionation was carried out as shown in table 3. About 27.41 % of acccumulated cadmium in the yeast cell was found in the precipitation fraction at 4,000 xg for 10min which is consisted mainly of cell debris, nucleus, etc.

In the precipitation fraction which obtained from above supernatant, 57.34% of cadmium was found, and 15.25% of it was assayedin the supernatant centrifugation at 16,000xg for 20 min. It is consisted that the precipitate is polysome, mitochondria, etc., and that the supernatant is microsome, cytosol, etc.

DISCUSSION

Cadmium-ion tolyant microorganisms were isolated by some investigators. *Klebsiella rhinoscleromatis* (2) took a tolerance of 1,000μg/ml of Cd^{++} in addition, *Pseudomonas aeruginosa* (1) and *Enterobacter cloaceae* (8) grew in the presence of 1,500μg/ml of Cd^{++} *Erwinia* sp. C-7 (4) grew in a medium containing Cd^{++} up to 2,800μg/ml.

Oda and Minami (2) divided Cd^{++} tolerant microorganism into three groups ; Cd^{++} sensitive strain, CSS (<5~5μg/ml), Cd^{++} moderate tolerant strain, CMTS, (50~400μg/ml), and Cd^{++} extreme tolerant strain, CETS, (700~1,000μg/ml).

The strain B-7 showed fairly good growth in a medium containing Cd^{++} up to 3,000μg/ml (Fig. 1) According to the classiffication by Oda (2), the strain B-7 yeast is a Cd^{++} highest extreme

tolerant strain (CHETS) in the heavy metal-ion tolerant microorganisms which were isolated by the other investigators (1, 2, 4, 8)

This strain B-7 grew very well at 20~22°C and the range of pH 5.0~8.0. The optimal pH for intracellular accumulation of cadmium was also indicated 6.0 (Fig. 2). The good growth of the strain B-7 was appeared at wide range of pH from 5.0 to 8.0, but the intracellular accumularation of cadmium was strongly inhibited at other pH ranges except for optimal pH 6.0.

The intracellular accumulation of cadmium was rapidly increased during the second half of stationary phase and the maximum accumulation was found to require longer time than the time which reached to maximum cell growth (Fig. 3).

An antifoamer, Silicone KM-70, did not affect a growth of the strain, B-7, whereas activated intracellular accumulation of cadmium (Table. 2). From the results, accumulatiotn of cadmium into the yeast cell was accelerated by the antifoamer. We suggest that a permeability of cell membrane was affected by antifoamer.

The maximum intracellular accumulation of cadmium was measured to be 34.17mg/g dried cells during cultivation in medium containing 500μg/ml of cadmium-ion and 0.2% of antifoamer under aerobic condition at 28°C for 48 hours (Table. 1).

Horitsu *et al* (9) reported that about 88% of intracellular cadmium was found in the cytoplasm and about 12% was accumulated in the cell envelope fraction. Also 80% of cadmium was presented in the cytoplasm of E. coli (6). However, Kim *et al* (8) observed that approximate 70% of cadmium was measured in the cell wall fractcon of *Enterobacter cloace* Their results are contrary to each other. It is found that 73% of cadmium was distributed in the cytoplasm of the cadmiumion tolerant yeast cells.

ACKNOWLEDGEMENTS

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要 約

카드뮴에 오염될 가능성이 있는 아연 광산지역의 汚泥로 부터 重金屬耐性菌을 분리한 高度카드뮴耐性 酵母인 B-7 배양조건 및 균체내 카드뮴의 축적에 대하여 검토했다.

酵母 B-7은 3,000μg/ml의 카드뮴 함유배지에서 생육이 가능하며 다른 연구자들의 카드뮴耐性菌보다 耐性이 가장 큰 高度카드뮴耐性菌이었다.

이 酵母는 22~22°C 및 pH5.0~8.0의 조건에서 생육이 양호하였다.

카드뮴은 균체내 축적은 생육최적 pH인 6.0에서 가장 양호했으나, 그 이의의 pH에서는 급격히 저해되었으며 소포제 Silicon KM-70에 의하여 46.5%의 카드뮴 축적을 촉진시켰다.

카드뮴의 균체내 축적은 정지기 후반에 일어나며 0.2% Silicon과 500μg/ml의 카드뮴을 함유하는 배지에서 28°C, 48시간 진탕배양한 바 건조균체당(g) 34.17mg의 카드뮴을 축적시켰다.

축적 카드뮴의 73%가 세포질 중에 축적되었으며 나머지 27%의 카또뮴은 세포벽 및 핵과 같은 4,000×g에서 침전되는 성분에 축적되었다.

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