KOR. JOUR. MICROBIOL. February. 1986 p. 57-61 Vol. 24, No. 1

Characterization of a Cadmium-Ion Tolerant Strain of *Hansenula anomala*

Yu, Tae-Shick, Hyung-Ik Song* and Ki-Taek Chung**

Department of Biology, College of Natural Science, Keimyung University, Taegu, Korea

**Department of Food Technology, Taegu Technical Junior College, Taegu, Korea*

***Department of Food Science and Technology, College of Agriculture, Kyungpook National University, Taegu, Korea*

카드뮴 내성 *Hansenula anomala* 균주의 특성

俞大植 · 宋亨翼* · 鄭基澤**

계명대학교 자연과학대학 생물학과 *대구공업전문대학 식품공업과

**경북대학교 농과대학 식품가공학과

In order to remove of cadmium from waste water an identification of a cadmium-ion tolerant yeast B-7 isolated from the sludge of zinc mining district was studied. By the taxonomical characteristics of strain B-7 it was identified as *Hansenula anomala* B-7 or similar strain. The cadmium-ion tolerance of the strain B-7 was determined as 2,700 μg/ml of cadmium-ion by density gradient agar plate method. The strain B-7 grew well in an aqueous medium containing 1,000 μg/ml of cadmium-ion.

Since it was found that Itai-itai disease in human body was caused by cadmium accumulation largely due to environmental pollution, cadmium as an environmental pollutant has recently become the subject of social problem. A cadmium-ion tolerant microorganisms might be useful for the removal of cadmium from waste water. Recently studies for this purpose were initiated.

Several cadmium-ion tolerant bacteria have been reported (Horitsu et al., 1974; 1979; Kim et al., 1976; Oda et al., 1978; Yu, 1979; Yu et al., 1980). But it is known that a cadmium-ion tolerant yeast is very difficult to isolate, because cadmium is highly toxic to eucaryotic cell, especially to yeast cell. Tohoyama and Murayama (1977) have obtained cadmium resistant yeast strains by the repeated training of the parent strain with cadmium-contained media. But the isolation of naturally occuring cadmium-ion tolerant yeast has not been reported.

In previous studies (Yu, 1979; Yu et al., 1980), a high cadmium-ion tolerant microorganisms, strain B-7 and C-7, were successfully isolated in zinc mining districts. A cadmium-ion tolerant bacterium C-7 was identified *Erwinia* sp., meanwhile the strain B-7 was a cadmium-ion tolerant yeast.

A silicone-based antifoamer did not inhibited the growth of the strain B-7, but rather stimulated the intracellular accumulation of cadmium (Yu et al., 1981[a]; 1981[b]). One gram of the strain B-7 cells accumulated 34.17 mg of cadmium when the strain was grown in aqueous medium containing 500 μg/ml of cadmium-ion and 0.02 vol % of an-

tifoamer at 28°C with shaking for 48 hours. About 73% of cadmium taken up by the cell was found in the cytoplasm.

In the precent paper, we report that the cadmium-ion tolerant yeast B-7 is identified as *Hansenula anomala.*

MATERIALS AND METHODS

Isolation of Cadmium-Ion Tolerant Yeast

Cadmium-ion tolerant yeasts were isolated from the sludge of some areas (zinc mining companys, industrial complexes, etc.) located in Korea with a fair chance of cadmium pollution. The yeast was isolated in the medium consisted of 10g of glucose, 10g of peptone, 5g of yeast extract and 500mg of cadmium-ion in 1,000ml of deionized water, and PH was adjusted to 6.0. The cells were grown in test tube (1.5 × 15 cm) containing 5 ml of medium at 30°C for 3 days.

After being incubated, the isolation of cadmium-ion tolerant yeast was performed by the agar plate method. The purity of the isolated yeast was determined by observations under the microsope.

Identification of the Isolated Yeast

Morphological and physiological studies of the isolated yeast were carried out according to Iizuka and Goto (1977), and Hasegawa (1975). And taxonomical studies were carried out according to the classification of Barnett and Pankhurst (1974), and Lodder (1971).

Cultivation

The basal medium contained 10g of glucose, 10g of peptone, 5g of yeast extract, 1g of NaCl, 0.3g of $MgSO_4$ $7H_2O$, 0.1g of KH_2PO_4 and 0.1g of K_2HPO_4 in 1,000ml of deionized water, and PH was adjusted to 6.0. The cells were grown in a 500ml Sakaguchi shaking flask containing 100ml of media at 25°C on a reciprocal shaker (120 strokes/min., amplitude 7 cm). Cell growth was determined by measuring the optical density of broth at 660 nm and dried cell weight(g).

Datermination of Cadmium-Ion Tolerance

The tolerance test of cadmium-ion was carried out by density gradient agar plate method described by Szybalski and Bryson (1952). A petri dish (10 × 1.5 cm) with a linear cadmium gradient agar plate containing 3,500 μg/ml of cadmium-ion on one side and 350 μg/ml of cadmium-ion on the opposite side was prepared. The strain B-7 was precultured in basal madium without cadmium for 24 hours before assaying its tolerance. The cell suspension suitably diluted (ca. 10^{-4} cells/ml) was streaked, and the growth range on the plate was measured after 7 days incubation at 25°C.

Chemicals

Cadmium compounds used in this study were cadmium nitrate, $Cd(NO_3)_2$ $4H_2O$, which was manufactued from Hayashi Pure Chemical Co., Japan. All other chemicals used were guaranteed reagent.

RESULTS AND DISCUSSION

Isolation and Screening of Cadmium-Ion Tolerant Yeast

Seventeen strains of microorganisms on basal media containing 1,000 μg/ml of cadmium-ion were isolated from sludge obtained from five zinc mining companies. Among these strains two strains (B-7, C-7) were very easily grown on agar plate containing 2,000 μg/ml of cadmium-ion. Of the two, the strain B-7 was isolated from sludge from Sanmak Zinc Mining Co. located at Kalsan-ri, Zaesan-myun, Bongha-kun, Kyung Sang Pook-

Table 1. *Morphological and Cultural Characteristics of the Strain B-7.*

Morphological properties	
Cell shape	oval, ellipsoidal
Cell size(μ)	5.3×6.1
Ascospore number and shape	2-4, hat shape
Pseudomycelium formation on corn meal agar	positive
Pellicle formation	positive (heavy)
Budding	multilateral
Cultural properties	
Growth on the streak culture	continuous with even edge
Colony profile	convex
Colony periphery	undulate

Table 2. *Physiological Properties of the Strain B-7*

Fermentation			
Glucose	+	Lactose	−
Galactose	−	Melibiose	+
Sucrose	+	Raffinose	+ (1/3)
Maltose	−	Glycerol	+
Assimilation of carbon compounds			
D-Arabinose	−	Inositol	−
Glucose	+	Soluble starch	+
Galactose	−	Inuline	−
L-Rhamnose	−	Salicin	+
Sucrose	+	Glycerol	+
Maltose	+	Ethanol	+
Lactose	−	Cellobiose	+
Melibiose	−	Erythritol	+
Raffinose	+	Succinate	+
D-Mannitol	+	Citrate	+
Assimilation of potassium nitrate			positive
Production of esters			positive
Growth in vitamin-free medium			positive
Sodium chloride tolerance			>=17%, but <=18%

Do, Korea, and this strain was a yeast. The other strain C-7 was a bacterium (Yu, 1979; Yu et al., 1980).

Taxonomical Studies of the Strain B-7

Cadmium-ion tolerant microorganism B-7 was identified in order to determine the taxonomical position of the cadmium-ion tolerant microorganism. The morphological and cultural characteristics of the strain B-7 are shown in Table 1. The strain B-7 reproduced by multilateral budding was 5.3 × 6.1 μm in size, oval or ellipsoidal in shape. The strain B-7 formed ascospore and weakly formed psudomycellia on corn meal agar. But the typical yeast cell was easily changed to psudomycellial cell by the presence of 1,000 μg/ml of cadmium-ion. The physiological properties of the strain B-7 were shown in Table 2. As for taxonomical characteristics, this strain B-7 was oval or ellipsoidal on shape, its assexual reproduction was done by multipolar budding, its nitrate growth test was positive, and it produced from two to four ascospores. From these characteristics it was concluded that the strain B-7 belongs to the *Hansenula*. The key characteristics in the survey of the species of the genus *Hansenula* were as follows: positive D-glucose fermentation test, negative melibiose growth test, positive soluble starch and maltose growth test, negative inuline growth test, positive no vitamine growth test, negative L-rhamnose growth test, positive sucrose fermentation test, negative maltose fermentation test, positive production of esters, and a good growth in 10% sodium chloride. From those characteristics, the isolated cadmium-ion tolerant microorganism was identified as *Hansenula anomala* B-7 or similar strain by Barnett and Pankhurst (1974), or *Hansenula anomala* var. *anomala* B-7 or similar strain by Lodder (1971).

Cadmium Tolerance of the Yeast B-7

According to a density gradient agar plate method by Szybalski and Bryson (1952), cadmium-ion tolerance of the strain was determined as 2,700 μg/ml of cadmium-ion, as shown in

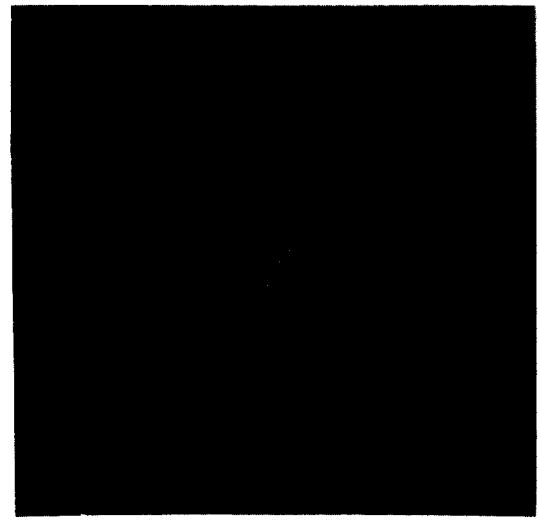

Fig. 1. *Determination of Cadmium-ion Tolerance of the Strain B-7. The cell suspension of the strain B-7 was streaked on a gradient agar plate which was layered in two concentrations, containing 3,500 μg/ml of cadmium-ion on right side and 350 μg/ml of cadmium-ion on the left side of the disk. The strain B-7 was grown at 25°C for 7 days.*

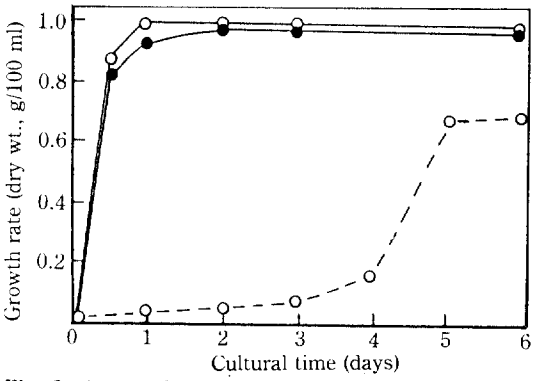

Fig. 2. *Growth Curves of the Strain B-7 at the Various Cadmium Concentration.*
—○—; none,
—●—; 100 μg/ml,
--○-; 1,000 μg/ml of cadmium ion.

Fig. 1. This cadmium-ion tolerant yeast B-7, *Hansenul anomala,* had higher cadmium-ion tolerancy than that of cadmium-ion tolerant microorganisms isolated by previous investigations (Horitsu et al. 1974; 1979; Kim et al., 1976; Oda et al., 1978; Tohoyama et al., 1977). The maximum cell growth in aqueous medium without cadmiumion was obtained under an aerobic condition at 25°C for 12 hours. Furthermore, the yeast showed a very good growth in aqueous medium containing 1,000 μg/ml of cadmium-ion. But in the presence of 1,000 μg/ml cadmium-ion a lag phase of the strain B-7 was lengthened for 4 days and thereafter showed 65% level of the population of the cells for 7 days compared with no cadmium-ion under the same conditions, as shown in Fig. 2.

Optimum Temperature and pH on Growth

The strain B-7 yeast grew very well at 20–22°C, and optimum initial PH was found to be near 6.0.

Effect of Various Heavy Metallic Ions

The tolerance of the strain B-7 was assayed in medium containing 500 μg/ml of each heavy metallic ions ($CdCl_2 \cdot 2\frac{1}{2}H_2O$, $HgCl_2$, $ZnCl_2$, $CuCl_2 \cdot 2H_2O$, $SnCl_2 \cdot 2H_2O$, $PbCl_2$). The growth of this strain B-7 was not inhibited by heavy metallic ions except for mercury ion. Mercury strongly inhibited its growth at 100 μg/ml with about 95% of growth inhibition rate, as shown in Table 3. In the presence of 10 μg/ml of mercury ion, inhibition rate of growth was found to be about 50% compared with control. The growth of the strain B-7 was not affected by various cadmium compounds (chloride, nitrate, acetate, sulfate), as shown in Table 4.

Table 3. *Effect of Heavy Metal Ions on the Growth.*

Metal ions[a]	Relative growth (%)	Final PH
Cd^{+2}	110.3	6.9
Hg^{+2} [b]	5.7	6.1
Zn^{+2}	98.0	6.9
Pb^{+2}	100.0	7.1
Cu^{+2}	101.9	6.4
Sn^{+2}	100.9	7.0
None	100.0	7.0

The strain B-7 was grown at 25℃ for 24 hours by shaking in the aqueous medium with various metallic ions, respectivelly.
a) as chloride salts
b) broth with 100 μg/ml of mercury ion.

Table 4. *Effect of Cadmium Compounds on the Growth.*

Cadmium compound (Cd^{+2}, 500 μg/ml)	Relative growth (%)	Final PH
Cadmium chloride	94.2	6.9
Cadmium nitrate	94.6	6.9
Cadmium acetate	91.5	7.0
Cadimum sulfate	100.3	6.9
None	100.0	7.0

The strain B-7 was grown at 25℃ for 24 hours by shaking.

摘 要

폐수중에 함유된 카드뮴을 미생물학적인 방법으로 정화하기 위한 기초 연구로서 아연 광산에서 고도 카드뮴 내성 효모 B-7을 분리하여 동정한 바 ***Hansenula anomala*** B-7 또는 그 유연균으로 밝혀졌다.

이 효모는 농도 구배 한천 평판법에 의하여 2,700μg/ml의 카드뮴 농도에서도 내성을 나타내므로 고도 카드뮴 내성균으로 분류되었고 1,000μg/ml의 고 농도 카드뮴 함유 액체 배지에서도 생육이 가능함을 확인하였다.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Seu, Jung Heun, faculty of Natural Science College, Kyung Pook National University for helpful discussions and suggestions for the identification of the isolated microorganism.

REFERENCES

1. Barnett, J.A. and R.J. Pankhurst, 1974. A new key to the yeasts. North-Holland Publishing Co., Amsterdam, pp 138-139.
2. Hasegawa, T., 1975. Taxonomy and identification of microorganisms. Tokyo University Press, Tokyo, Japan, pp 67-137.
3. Horitsu, H., H. Kato and M. Tomoyeda, 1979. Uptake of cadmium by a cadmum chloride-tolerant bacterium, *Pseudomonas aeruginosa, J. Ferment. Technol.*, **57,** 273-279.
4. Horitsu, H., T. Maeda and M. Tomoyeda, 1974. Isolation of a cadmium nitrate-tolerant microorganisms and the incorporation of the heavy metal ion into the cells of the microorganism. *J. Ferment. Technol.*, **52,** 14-19.
5. Iizuka, H. and S. Goto, 1977. Taxonomy and identification method of yeasts, 2nd ed. Tokyo University Press, Tokyo, Japan, pp 1-126.
6. Kim, Y.B. and S.R. Lee, 1976. Isolation of cadmium-tolerant bacteria and accumulation of cadmium into the bacterial cell. *Korean J. Appl. Microbial. Bioeng.*, **4,** 111-115.
7. Lodder, J., 1971. The yeasts, a taxonomic study, 2nd ed. North-Holland Publishing Co., Amsterdam, pp 247-249.
8. Oda, M. and K. Minami, 1978. Isolation and identification of the cadmium ion-tolerant microorganisms and accumulation of cadmium-ion by the cells. *J. Ferment. Technol.*, **56,** 1-8.
9. Szybalski, W. and V. Bryson, 1952. Genetic studies on microbial cross resistance to toxic agents, I. Cross resistance of *Escherichia coli* to fifteen antibiotics. *J. Bacteriol.*, **64,** 489-499.
10. Tohoyama, H. and T. Murayama, 1977. Isolation and some characteristics of cadmium-resistant yeast. *Agric. Biol. Chem.*, **41,** 1523-1524.
11. Yu, T.S., 1979. Microbial characteristics of heavy metal ion tolerant microorganisms. *Korean J. Appl. Microbial. Bioeng.*, **7,** 183-190.
12. Yu, T.S., M.H. Kim and H.I. Song, 1980. Nutritional and cultural characteristics of cadmium-ion tolerant microorganism, *Erwinia* sp., C-7. Keimyung University, *KwaHakNon-Jip,* **6,** 41-49.
13. Yu, T.S and M.H. An, 1981[a]. The effect of cadmium on the growth of *Hansenula anomala* var. *anomala. J. Institute of Natural Science,* **1,** 17-21.
14. Yu, T.S. and H.I. Song, 1981[b]. Cultural conditions of heavy metal-ion tolerant microorganism and accumulation of heavy metal-ion into the cells. *Korean J. Appl. Microbial. Bioeng.*, **9,** 59-64.

(Received Dec. 11, 1985)