Isolation of Potassium Chromate-resistant Bacterium and Reduction of Hexavalent Chromium by the Bacterium

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Summary

A bacterial strain highly resistant to potassium chromate was isolated from chromium sludge, and was found to belong to the genus *Pseudomonas* and named *Pseudomonas* K-21. The growth of the bacterium was attained even in the presence of 20,000 ppm potassium chromate, whereas the onset of growth was significantly delayed and the cells somewhat elongated, This value was approximately 100 times higher than that obtained with other bacteria. When *Pseudomonas* K-21 cells were allowed to react with potassium chromate in the presence of glucose, hexavalent chromium disappeared and trivalent chromium appeared in the reaction mixture. In cells of *Pseudomonas* K-21, the possible existence of enzymes which catalyze reduction of heaxvalent chromium was suggested.

Introduction

Enviromental pollution by hexavalent chromium has become one of the greatest social problems in Japan. Recently, Venitt *et al.*[1-3] reported that hexavalent chromium is a strong mutagen for bacteria, while trivalent compounds are neither toxic nor mutagenic for them. In many cases, mutagen are known to be carcinogen. Therefore, it is important to remove hexavalent chromium from circumstances heavily contaminated by it. The authors initiated the work in order to remove hexavalent chromium by the use of chromium-resistant microorganism. The present paper deals with isolation and taxonomic observation of a chromium-resistant bacterium and reduction of hexavalent chromium by this organism.

Materials and methods

Chromium compounds

Hexavalent chromium was tested as potassium chromate (K_2CrO_4) and potassium dichromate ($K_2Cr_2O_7$). Trivalent chromium was tested as chromium chloride ($CrCl_3 \cdot 6H_2O$).

Isolation and cultivation of K_2CrO_4-resistant bacterium

Isolation of K_2CrO_4-resistant bacterium was performed with agar-plate technique, and cultivation of isolates was conducted in flasks with or without shaking at 30°C. The composition of basal medium used for the above procedures was as follows: Polypepton 5 g, meat extract 5 g, NaCl 5 g, glucose 5 g, and distilled water 1,000 m*l*, pH 7.0. Chromium compound, dissolved in distilled water and sterilized, was added to the basal medium at a definite concentration prior to inoculation of test organisms.

Identification of K_2CrO_4-resistant bacterium

Taxonomic study of a selected K_2CrO_4-resistant bacterium was carried out according to Buchanan *et al.*[4] and Komagata[5].

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Measurement of chromium

Chromium was determined spectrophotometrically using diphenylcarvazid[6].

Results and discussion

1. Isolation and identification of hexavalent chromium-resistant bacterium.

Hexavalent chromium is known to be toxic for various bacteria at low concentration[7]. The result of preliminary experiment using *Bacillus subtilis*, *Echerichia coli*, and *Protes vulgaris* indicated that the growth of these bacteria was allowed in the presence of potassium chromate less than 200 ppm. We tried isolation of potassium chromate-resistant strains in the presence of 800 ppm K_2CrO_4, and, as a result, 185 strains of resistant bacteria were obtained from waste of chromium plating industry and

Table 1. Number of Cr-Resistant Strains with Respect to Concentration of K_2CrO_4.

Concentration of K_2CrO_4 (ppm)	Number of resistant strains
800	185 (100%)
1500	133 (71.9%)
3000	31 (16.8%)
10000	3 (1.6%)

Agar media.

Table 2. Taxonomical studies of the Chromium-resistant Bacterium.

MORPHOLOGICAL CHARACTERS
- Shape and size: short rod, rounded end, $0.5 \times 1.5\,\mu$, occurring singly
- Flagella: motile with a single polar flagellum
- Gram reaction: negative
- Growth: aerobic

CULTURAL CHARACTERS
- Nutrient agar colonies: circular, smooth, convex, entire, translucent, pigment is not produced
- Slant culture: abundant, spreading growth
- Stab culture: Filiform
- Broth culture: Marked turbidity with ring and heavy sediment

PHYSIOLOGICAL CHARACTERS
- Gelatin: rapid liquefaction
- Milk clotting: enzymatic
- Indol: not produced
- Hydrogen sulfide production: positive
- Ammonia production: negative
- Voges-Proskauer reaction: positive
- Methyl red test: negative
- Starch: not hydrolyzed
- Catalase: positive
- Urease: negative

from soil. These isolates were then tested for their capability of growth in media of higher K_2CrO_4 concentrations. It is seen from the data presented in Table 1 that number of strains which could grow in the presence of 1,000 ppm K_2CrO_4 was only 3. Trials of successive adaptation to higher concentration of potassium chromate were followed using these three strains, and K-21 strain, which attained growth in the presence of 20,000 ppm K_2CrO_4, was finally selected as the most chromium-resistant becterium and used in the following experiments. The bacterium seems to be much more tolerant to chromium than *Pseudomonas aeruginosa*[8] and *P. ambigua* G-1[9] so far reported.

K-21 strain was $0.5 \times 1.5\,\mu$ in size, rod shape and had polar flagellum. Table 2 summarized the morphological and physiological characteristics of the bacterium. On the basis of these observations, this bacterium was found to belong to the genus *Pseudomonas* and named *Pseudomonas* K-21.

2. *Resistance of the organism to chromium*

Resistance of the organism to bactericidal action of chromium was studied using three kinds of chromium compounds. As shown in Fig. 1, the organism was markedly resistant to $CrCl_3$ (trivalent, pH 5.0) and K_2CrO_4 (hexavalent, pH 7.0) as compared with $K_2Cr_2O_7$ (hexavalent, pH 5.0) which exerted powerful killing effect on the bacterium. The growth curves of *Pseudomonas* K-21 in media containing various amounts of K_2CrO_4 were shown in Fig. 2. The rate of growth of this organism decreased with increasing concentration of K_2CrO_4 up to 350 ppm. Elongation of cells were observed when the organism was grown in the presence of more than 1,000 ppm K_2CrO_4.

3. *Decrease of hexavalent chromium during cultivation of the organism*

The organism was inoculated to the basal medium containing 200 ppm K_2CrO_4 and different amount

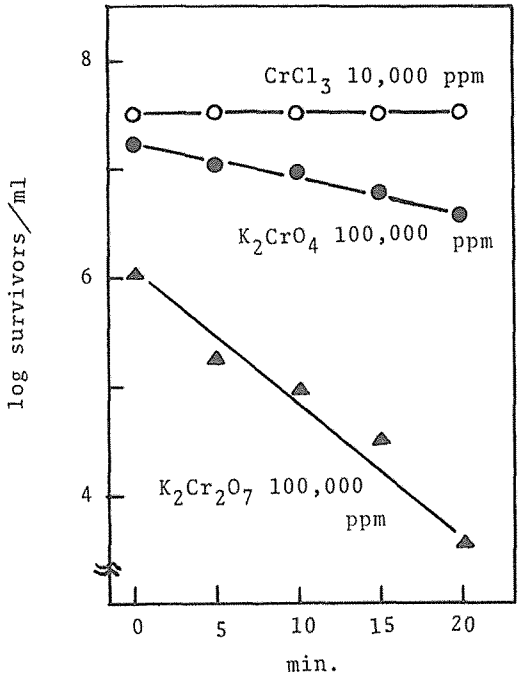

Fig. 1. Comparative killing curve.

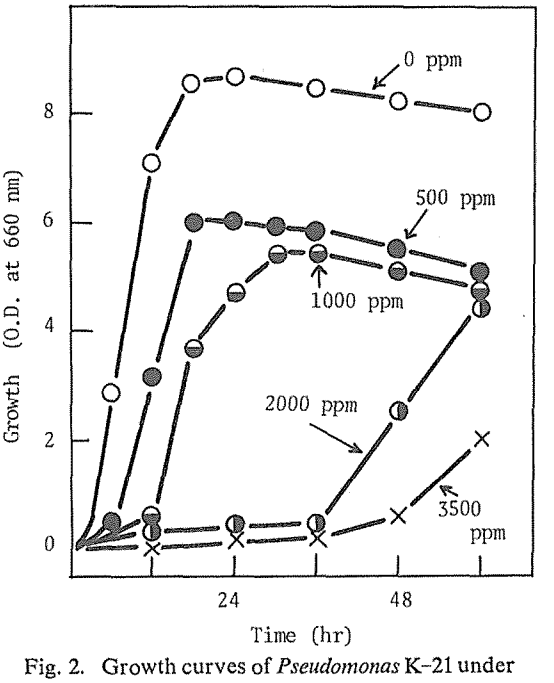

Fig. 2. Growth curves of *Pseudomonas* K-21 under different K_2CrO_4 concentrations.

The organism was cultivated in the basal medium containing different amount of K_2CrO_4 on a shaker at 30°C.

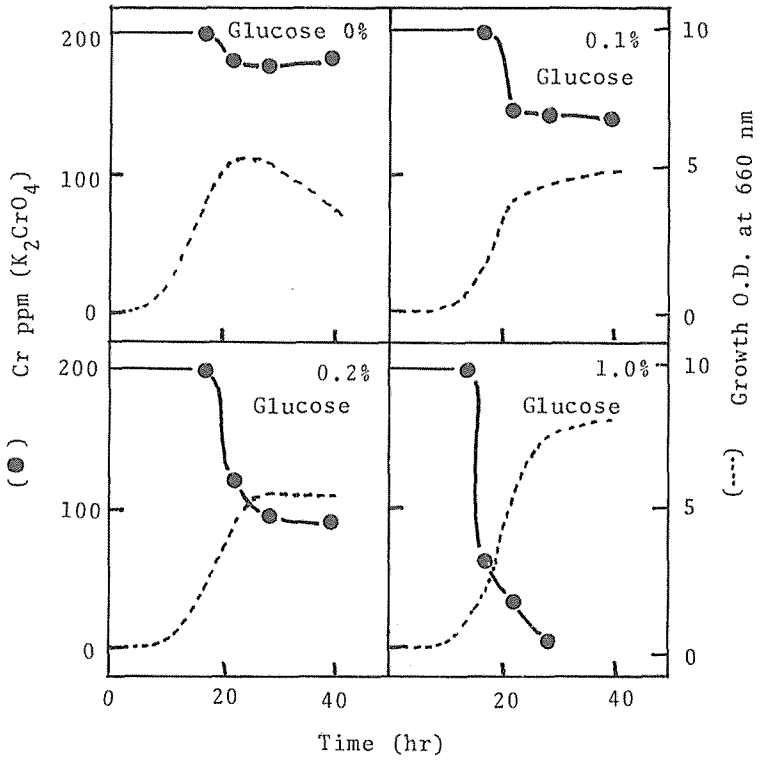

Fig. 3. Effect of glucose concentration on disappearance of hexavalent chromium.

The organism was cultivated in the basal medium containing different amount of glucose and 200 ppm K_2CrO_4 on a shaker at 30°C.

of glucose and cultivated on a shaker at 30°C. At intervals the culture fluid was withdrawn and analyzed for residual hexavalent chromium, cell growth being also determined as optical density at 600 nm. It was found that decrease of hexavalent chromium in the medium occurred along with cell growth and the rate of decrease was markedly stimulated by increasing amount of glucose added to the medium (Fig. 3.).

4. Decrease of hexavalent chromium by cells of the organism

Cells, harvested by centrifugation from 48-hr-old culture of *Pseudomonas* K-21 grown in the basal medium, was resuspended in a supernatant of the above culture to prepare cell suspensions of different concentration, potassium chromate being added at a concentration of 200 ppm. Each sample (2 m*l*) was incubated at 40°C and, at intervals, residual hexavalent chromium was analyzed. The results shown in Fig. 4 indicate that the decreased amount of hexavalent chromium was approximately proportional to the cell concentration. However, no decrease of hexavalent chromium was observed when the samples of the above experiment were incubated at 0°C instead of 40°C, or cells were treated by heat (80°C), acid or alkali prior to incubation. It was also found that disappearance of hexavalent chromium was accompanied with appearance of trivalent chromium during incubation of the sample. These facts strongly suggest that there may exist, in cells of this bacterium, certain enzymes which catalyze the reduction of hexavalent chromium to form trivalent chromium.

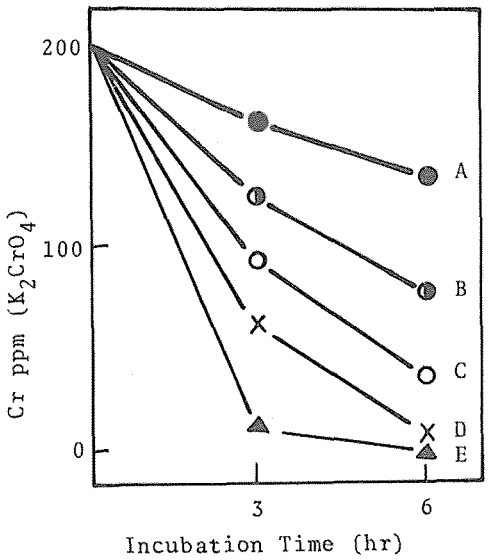

Fig. 4. Effect of cell concentration on decrease of hexavalent chromium.
Cell concentration: A no cell, B 2×10^9 cell/m*l*, C 4×10^9 cell/m*l* (corresponded to original culture), D 8×10^9 cell/m*l*, E 1.6×10^{10} cell/m*l*.

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摘　　要

クロム酸カリ耐性細菌の分離と本細菌による六価クロムの還元

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クロム酸カリに耐性を有する細菌をクロムスラッジから分離し，この菌がシュードモナス属の細菌であることを明らかにし，シュードモナス K-21 と命名した。この菌の生育は 20,000 ppm のクロム酸カリの存在下でも認められるが，生育はかなり遅れ，細胞が幾分長大化した。この耐性は他の普通の細菌の約 100倍に相当した。このシュードモナス K-21 をブドー糖の存在下でクロム酸カリに反応させると，六価クロムが消失して三価クロムが反応混合物中に現れた。シュードモナス K-21 の細胞中に，六価クロムの還元を触媒する酵素の存在の可能性が考えられた。