P706

Cloning of hexavalent chromium reductase gene from *E.coli* ATCC 33456

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Abstract

E.coli ATCC 33456 has relatively higher activity of Cr(VI) reduction than other microorganism. The purpose of this research is cloning of Cr(V) reductase from *E.coli* ATCC 33456. Using colony and southern hybridization, we selected two condidates. Among candidates, pNCR9 is higher Cr(VI) reduction activity than *E.coli* ATCC 33456. Purified Cr(VI) reductase antibody was reacted at estimated 42Kda protein band of candidate's crude extract on 12% SDS-PAGE. This results showed cloned gene's product is very similar to purified Cr(VI) reductase from *E.coli* ATCC 33456.

Key words: *E.coli* ATCC 33456, Cr(VI) reduction

Introduction

Cr(VI)(chromate) is a widespread industrial and nuclear waste. At the Department of Energy(DOE) sites, for example, it is the second most common heavy metal contaminant, ranging in concentration between 0.008 to 173 μM in groundwater and 98nM to 76mM in soil and sediments.[3] Chromate is toxic, mutagenic, and probably carcinogenic.[4]

The purpose of this study is cloning of Cr(VI) reductase from *E.coli* ATCC 33456, as known this strain has relatively high activity of Cr(VI) reduction.[1]

Materials and Methods

1. Isolation of Cr(VI) reductase gene

After extraction of genomic DNA from *E.coli* ATCC 33456, genomic DNA is digested partially by *Kpn*I. DNA digests were electrophoresed on 0.8% agarose gel at 1.5V cm^{-1}. DNA fragments of 4-6Kbp size were sliced out from the gel. The fragments in each fraction were purified by Mega bead^{TM} agarose gel extraction kit(Intron, KOREA). The DNA fraction was ligated to pBluescript

SK(+) treated *Kpn*I and CIAP(calf intestinal phosphatase). *E.coli* DH5α competent cells were transformed with the ligation mixture. Using MCrRI probe, candidates were selected by colony and southern hybridization.

2. Acquirement of crude extract from candidates

After extraction of recombinant DNA from candidates, *E.coli* BL21-SI competent cells(Gibco BRL, USA) were transformed with recombinant DNA of candidates. Until cell OD is 0.5, recombinant *E.coli* BL21-SI were incubated on Luria-Botani without NaCl(LBON) broth at 30℃. After NaCl solution was added to culture broth adjusted to 0.3M NaCl, the broth was incubated for 3hr. Cell pellet was isolation to culture broth by centrifugation. Cell pellet was resuspended to 50mM potassium phosphate(pH 7.0) and broken by sonicator(Branson, USA). Cell lysate was centrifuged to remove cell debris and unbroken cell at 12000g, 4℃ for 10min.

3. Measurement of Cr(VI) reduction activity

The Cr(VI) reducing activity in 19.2μM(1ppm) Cr(VI), 0.4mM NADH, 50mM potassium phosphate(pH7.0) solution was assayed after incubation at 37℃ for 180 min. All sample were duplicated. Activity of Cr(VI) reductase was assayed by measuring the decrease of Cr(VI). One unit of the Cr(VI) reductase was defined as the mg protein which decreased 1μM of Cr(VI) per minute.[3)]

4. SDS-PAGE and immuno-blotting

All method was followed to Protein methods.[2)]

Results and Discussions

Using MCrRI probe, as designed on the basis of N-terminal amino acid sequence of purified Cr(VI) reductase(unpublished) from *E.coli* ATCC 33456, MCrI probe was hybridized at estimated 3Kbp of DNA digests by *Kpn*I on agarose gel through genomic southern hybridization. We selected 2 candidates, as named pNCR2 and pNCR9, through colony and southern hybridization.

pNCR2 and pNCR9 of Cr(VI) reduction activity is higher activity than *E.coli* ATCC 33456.(Fig. 1) And purified Cr(VI) reductase antibody was reacted at estimated 42Kda protein band of candidate's crude extract on 12% SDS-PAGE.(Fig. 2) This results showed cloned gene's product is very similar

to purified Cr(VI) reductase from *E.coli* ATCC 33456.

요약

E.coli ATCC 33456은 6가 크롬 환원능이 있다고 보고되어진 미생물보다 비교적 높은 활성을 지닌다. *E.coli* ATCC 33456에서 6가 크롬 환원효소를 클로닝하기 위해서 colony hybridization과 southern hybridization을 통하여 2개의 형질 전환 균주를 획득할 수 있었다. 두 균주 모두 *E.coli* ATCC 33456이 가진 6가 크롬 환원능보다 약 2.5-4배정도 높은 활성을 보였으며, 두 균주의 조효소액을 가지고 immuno-blotting을 실시한 결과, *E.coli* ATCC 33456에서 분리 정제된 6가 크롬 환원효소의 항체가 약 42Kda에서 반응하는 것을 확인할 수 있었으며, 이 결과는 분리 정제된 6가 크롬 환원효소와 매우 유사한 유전자 산물이 두 균주에서 나온다는 것을 보여주었다.

참고문헌

1. 배우철, Characterization and Purification of Cr(VI) reductase from *Escherichia coli* ATCC33456(1998)、명지대학교 이학석사논문, 16

2. Daniel M. Bollag and Stuart J. Edelstein, Protein Methods(1991), 95-208

3. Riley, R. G., J. M. Zachara, and F. J. Wobber, Chemical contaminants on DOE lands and selection of contaminant mixtures for subsurface science research(1992), Report DOE/ER-0547T. U.S. Department of Energy, Washington, D.C

4. Wetterhahn, K. E., and J. W. Hamilton, Molecular basis of hexavalent chromium carcinogenicity: effect on gene expression(1989), Sci. Total Environ., Vol(86), 113-129

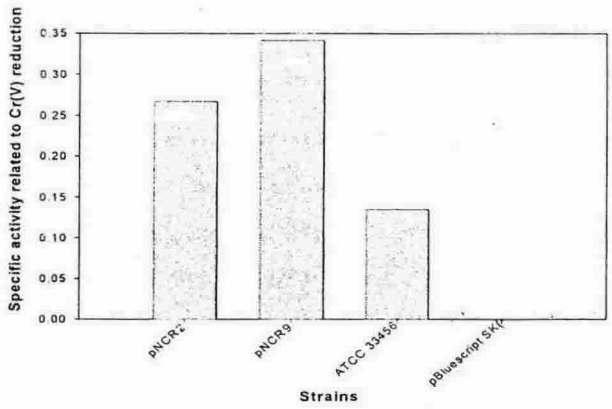

Figure 1. Cr(VI) Reduction activity Test

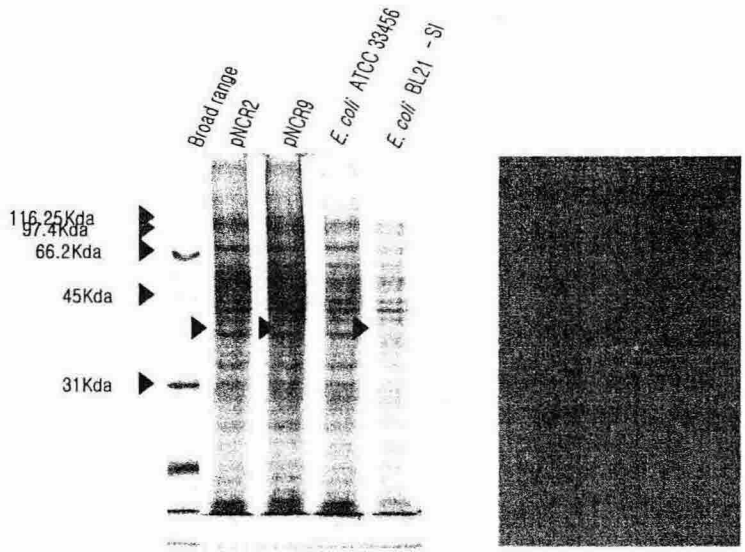

Figure 2. Immuno-Blotting