PLASMID MEDIATED CHROMATE RESISTANCE AND REDUCTION IN PSEUDOMONAS MENDOCINA MCM B-180

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Summary:

A chromate-resistant strain of Pseudomonas mendocina MCM B-180 capable of reducing hexavalent chromium was found to harbour a single plasmid. Incubation of the strain at 42°C for 24 h caused loss of chromate resistance as well as the plasmid, pARII80. Transformation of E. coli DH5α with purified pARII80 plasmid DNA resulted in simultaneous acquisition of resistance to chromate and the appearance of plasmid in the transformants. Most importantly, the plasmid transfer was found to confer chromate reduction ability on to the E. coli transformants.

Introduction:

Many bacteria belonging to genera Pseudomonas, Aeromonas, Enterobacter, Escherichia, Bacillus, Streptomyces etc. can reduce Cr^6+ to Cr^3+ (Cervantes and Silver, 1992). The phenomenon has attracted considerable interest owing to its potential use in the bioremediation of chromate-containing industrial waste waters. Recently, a microbiological method for the removal of hexavalent chromium from chromate-bearing cooling tower effluent was developed in our laboratory (Bhide et al., 1996). The process uses a strain of Pseudomonas mendocina MCM B-180 that is capable of reducing 2 mM Cr^6+ in 8 h with sugarcane molasses as nutrient. The P. mendocina strain used tolerated very high Cr^6+ concentrations (30 mM) in the milieu. However, chromate reduction could take place only at concentrations up to 2 mM. To investigate the genetic basis of chromate resistance and reduction in P. mendocina MCM B-180 the present studies were undertaken.

Materials and Methods:

Bacterial strains and growth media:

P. mendocina MCM B-180 was grown in EG medium (composition in g/l : NH₄Cl, 0.03; MgSO₄.7H₂O, 0.01; NaCl, 0.01; yeast extract, 0.15; peptone, 0.5; K₂HPO₄, 0.5; KH₂PO₄, 0.3; sodium acetate, 2.0. pH = 7.5) supplemented with 2 mM K₂CrO₄, unless specified otherwise. E. coli DH5α (Maniatis et al, 1982) strain used in this study was grown in EG medium containing glucose instead of sodium acetate.
Isolation of plasmid DNA:

Plasmid DNA was isolated by alkali lysis method and purified using Nucleobond® AX plasmid isolation and purification system (Macherey Nagel GmbH & Co., Germany), as described by the manufacturer.

Curing of chromate resistance phenotype:

Curing of chromate resistance phenotype was attempted with ethidium bromide (100 µg/ml), acridine orange (125 µg/ml) and by incubating the culture at 42°C for 24 h as described by Trevors (1986).

Transformation:

E. coli DH5α was transformed with purified plasmid DNA from P. mendocina MCM B-180 by CaCl2-heat shock treatment as described by Perbal (1984).

Plasmid stability:

P. mendocina was grown in Tryptone Yeast Extract (TYE) medium for 24 h. The total viable counts (TVC) of the culture were determined using both TYE agar and TYE agar + 2 mM chromate. Plasmid stability was expressed as percent ratio of TVC on (TYE + chromate) to (TYE).

Chromate reduction:

The chromate reduction ability of P. mendocina MCM B-180, cured strain MCM B-180C1, E. coli DH5α and E. coli DH5α(pARI180) transformants was compared in EG medium containing 0.2% glucose and 1 mM chromate. The cultures (ca. 109 cells) were inoculated in 100 ml medium in rubber stoppered bottles that were incubated at 30°C under stationary conditions for 24 h. The Cr6+ concentration was determined colorimetrically by diphenyl carbazide method (APHA, 1985). Chromate reduction efficiency of the cultures was expressed as percent difference in the Cr6+ concentrations at 0 h and after 24 h growth. Uninoculated EG medium containing 1 mM Cr6+ served as control.

Results and Discussion:

Plasmid DNA preparation of Pseudomonas mendocina MCM B-180 strain revealed the presence of a single plasmid (designated as pARI180). The molecular weight of the plasmid was approximately 32 kb (data not shown). Curing studies showed that ethidium bromide and acridine orange were unable to eliminate chromate resistance (CrR) phenotype in MCM B-180. However, incubation at 42°C for 24 h resulted in loss of chromate resistance at an efficiency of 92%. The agarose gel electrophoretic analysis of plasmid DNA preparation of the cured derivative revealed that loss of CrR phenotype in this strain was accompanied by the loss of plasmid pARI180 (Fig. 1) indicating possible involvement of pARI180 in chromate resistance in MCM B-180.