ABSTRACT

Analysis of putative regulatory and promoter regions of perchlorate degradation genes in Dechlorosoma sp. KJ operon

Perchlorate compounds have been used in the United States for more than 50 years, and estimates are that more than 10 million pounds of perchlorate containing waste has been disposed of in the environment. The perchlorate plume area is one of the worst hazardous waste sites on the full National Priority List.

Perchlorate compounds are chemically similar to the ubiquitous chloride ion, but two major differences exist: perchlorate is a strong oxidizing agent and anions of perchlorate are toxic to microorganisms, including humans. Consequently, removal of perchlorate from groundwater sources, which contain perchlorate as an incidental byproduct of military waste disposal, is a significant problem. There has been considerable research in the past decade to identify microorganisms that can remove perchlorate from groundwater by anaerobic metabolism of perchlorate to chloride (Coates and Achenbach, 2008). The research has primarily been focused on biochemical studies and the development of genetic tools for site-specific gene transfer to these novel perchlorate-degrading bacteria. In a previous study, we isolated a novel perchlorate-degrading bacterium that we named Dechlorosoma sp. KJ (Coates and Achenbach, 2008). We isolated the gene coding for chlorite dismutase (Cld) from this organism and cloned it into broad host range vector pCPP33 (strain RCB) (Coates and Achenbach, 2008). We successfully expressed Cld in E. coli and demonstrated the activity of Cld under anaerobic conditions. We also used this technology to verify the expression of recombinant proteins in diverse bacterial species and ultimately in naturally-occurring bacterial biofilms to facilitate bioremediation of perchlorate.

INTRODUCTION

Another diverse group of microorganisms belonging to four (of five) subclades of Perchlorate-Resistant Bacteria (PRB) have been identified to anaerobically grow on perchlorate and/or perchlorite as an electron acceptor. Perchlorite reducers were isolated from two major strategic sites, including transfite, a high level radioactive waste site, and the Savannah River Site, both in South Carolina, USA. These perchlorite reducers are further divided into novel genera, Acidipolis, Acinetobacter, Acinetobacter sp (formerly Dechloromonas) and Dechlorosoma sp. Dechlorosoma sp. (clade II).

PERCHLORATE REDUCTION BY DECHLOROSOMA SP. KJ

Perchlorate (chlorite) reduction is catalyzed by two enzymes, perchlorite reductase (Pcr) and chlorite dismutase (Cld). Chlorite dismutase is a homodimeric enzyme. The pcrABCD operon is further divided into novel perchlorate or perchlorite degradation genes from four different organisms (Coates and Achenbach, 2008). The operon consists of eight genes from all four organisms and is more closely related to each other than to previously published operons. Since, the sequence for Cld and Pcr were determined most closely related to each found in the Dechloromonas sp. and Sinorhizobium meliloti. The operon consists of a total of 14 genes, including the following genes: pcrA (encoding the NADH dehydrogenase subunit), pcrB (encoding a putative tetramerization domain), pcrC (encoding the PcrBC homodimeric protein), pcrD (encoding the chlorite dismutase), cld (encoding the chlorite dismutase), and pcrE (encoding a putative membrane-bound biocatalyst). The pcrABCD operon is encoded by the DNA of 1000 KJ DH5a/pET3a DNA of two previously published sequences DH5a/pET3a_D_cld and DH5a/pET3a_D_cld. The PcrABCD operon is found in perchlorate-reducing bacterium CR (strain RCB) (Coates and Achenbach, 2008). The pcrA gene was isolated from the gene is located di- stantly downstream of pcrABCD, where the gene is located upstream of the operon on the D. agitata chromosome. We are currently studying these genes and enzymes because we are hoping to develop an efficient in situ system for the remediation of perchlorite and/or chlorite in contaminated aquifers using naturally occurring microbial biofilms.

RESULTS AND DISCUSSION

We cloned and sequenced the chlorite-dismutase genes of two perchlorite (p-protobacteries) and two chlorite reducers (cyt-protobacteries). The nucleotide sequences were found to be more similar to each other than to any of the other previously reported chlorite dismutase genes sequences providing further evidence that this is a novel perchlorate-reducing bacterium. We have also used this technology to verify the expression of recombinant proteins in diverse bacterial species and ultimately in naturally-occurring bacterial biofilms to facilitate bioremediation of perchlorate.

We cloned and expressed the cld gene of Dechlorosoma sp. KJ in E. coli vector pET3a. A 28 kDa band was found to be over-expressed three hours after induction on the periplasmic fraction of E. coli BL21(pET3a-D cld). We excised the 28 kDa band and verified the identity of the protein as Cld using ESI-MS/MS analysis of tryptic peptides. The chlorite dismutase has been successfully expressed in E. coli DH5α cells, but not in E. coli BL21/pET3a-D cld. We excised the 28 kDa band and verified the identity of the protein as Cld using ESI-MS/MS analysis of tryptic peptides. The chlorite dismutase has been successfully expressed in E. coli DH5α cells, but not in E. coli BL21/pET3a-D cld. This material is based upon work supported by the US Army Corps of Engineers, Humphreys Engineering Center Support Activity under Contract No. W912HQ-07-C-0014.

Sample Preparation for Proteomic Analyses

A chromosomal library of Dechlorosoma sp. KJ was constructed using the pCR2.1 vector (Stratagene). The library was sub-screened with a 664 bp BamHI fragment, which encodes the chlorite dismutase gene, was excised from the plasmid DNA and subcloned into pET3a vector using the restriction sites BamHI and HindIII. A chromosomal library of Dechlorosoma sp. KJ was constructed using the pCR2.1 vector (Stratagene). The library was sub-screened with a 664 bp BamHI fragment, which encodes the chlorite dismutase gene, was excised from the plasmid DNA and subcloned into pET3a vector using the restriction sites BamHI and HindIII. The pCR2.1-D cld fragment was then further subcloned into the pET3a vector using the restriction sites BamHI and HindIII.

An expression vector pET3a-D cld was constructed using the restriction sites BamHI and HindIII. We excised the 28 kDa band and verified the identity of the protein as Cld using ESI-MS/MS analysis of tryptic peptides. The chlorite dismutase has been successfully expressed in E. coli DH5α cells, but not in E. coli BL21/pET3a-D cld. This material is based upon work supported by the US Army Corps of Engineers, Humphreys Engineering Center Support Activity under Contract No. W912HQ-07-C-0014.

Comparison of Genes involved in Perchlorate Biodegradation


Environmental Biotechnology Institute, University of Idaho, Moscow, ID 83844-1052

REFERENCES


Other Elements