World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:9, No:05, 2015

Aerobic Biodegradation of a Chlorinated Hydrocarbon by Bacillus Cereus 2479

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Abstract : Chlorinated hydrocarbon can be a major pollution problem in groundwater as well as soil. Many people interact with these chemicals on daily accidentally or by professionally in the laboratory. One of the most common sources for Chlorinated hydrocarbon contamination of soil and groundwater are industrial effluents. The wide use and discharge of Trichloroethylene (TCE), a volatile chlorohydrocarbon from chemical industry, led to major water pollution in rural areas. TCE is an mainly used as an industrial metal degreaser in industries. Biotransformation of TCE to the potent carcinogen vinyl chloride (VC) by consortia of anaerobic bacteria might have role for the above purpose. For these reasons, the aim of current study was to isolate and characterized the genes involved in TCE metabolism and also to investigate the in silico study of those genes. To our knowledge, only one aromatic dioxygenase system, the toluene dioxygenase in Pseudomonas putida F1 has been shown to be involved in TCE degradation. This is first instance where Bacillus cereus group being used in biodegradation of trichloroethylene. A novel bacterial strain 2479 was isolated from oil depot site at Rajbandh, Durgapur (West Bengal, India) by enrichment culture technique. It was identified based on polyphasic approach and ribotyping. The bacterium was gram positive, rod shaped, endospore forming and capable of degrading trichloroethylene as the sole carbon source. On the basis of phylogenetic data and Fatty Acid Methyl Ester Analysis, strain 2479 should be placed within the genus Bacillus and species cereus. However, the present isolate (strain 2479) is unique and sharply different from the usual Bacillus strains in its biodegrading nature. Fujiwara test was done to estimate that the strain 2479 could degrade TCE efficiently. The gene for TCE biodegradation was PCR amplified from genomic DNA of Bacillus cereus 2479 by using todC1 gene specific primers. The 600bp amplicon was cloned into expression vector pUC I8 in the E. coli host XL1-Blue and expressed under the control of lac promoter and nucleotide sequence was determined. The gene sequence was deposited at NCBI under the Accession no. GU183105. In Silico approach involved predicting the physico-chemical properties of deduced Tce1 protein by using ProtParam tool. The tce1 gene contained 342 bp long ORF encoding 114 amino acids with a predicted molecular weight 12.6 kDa and the theoretical pI value of the polypeptide was 5.17, molecular formula: C559H886N152O165S8, total number of atoms: 1770, aliphatic index: 101.93, instability index: 28.60, Grand Average of Hydropathicity (GRAVY): 0.152. Three differentially expressed proteins (97.1, 40 and 30 kDa) were directly involved in TCE biodegradation, found to react immunologically to the antibodies raised against TCE inducible proteins in Western blot analysis. The present study suggested that cloned gene product (TCE1) was capable of degrading TCE as verified chemically.

Keywords : cloning, Bacillus cereus, in silico analysis, TCE
Conference Title : ICBBB 2015 : International Conference on Bioscience, Biochemistry and Bioinformatics
Conference Location : Paris, France
Conference Dates : May 18-19, 2015