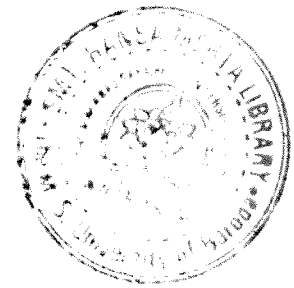

ABSTRACT

Pesticide biodegradation by soil bacteria

Harsukh Gopalbhai Tank

Department of Microbiology and Biotechnology Centre, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara-390002, Gujarat, India.

Gamma-Hexachlorocyclohexane (γ-HCH) degrading *Shewanella* sp. CGR-L1 and *Sphingobium* sp. CGR-L2 were isolated from industrial site soil (Baroda district, Gujarat, India) by direct isolation (without enrichment) and enrichment method (using γ-HCH as a sole source of carbon), respectively. γ-HCH degradation by *Shewanella* sp. CGR-L1 was confirmed by γ-HCH clearance assay, dehalogenase enzyme activity assay, HPTLC and GC-MS. GC-MS analysis confirmed the presence of γ-PCCH and 1,2,4-TCB, which are produced during the biodegradation of γ-HCH. Further, *linA* gene was amplified using primers specific for the conserved sequences. Sequence analysis of *linA* gene from *Shewanella* sp. CGR-L1 revealed 97% identity to *linA* gene of *S. japonicum* UT26 and *linA2* gene of *S. indicum* B90. Southern blot analysis revealed presence of four copies of the *linA* gene in the isolate *Shewanella* sp. CGR-L1. Plasmid curing and PCR analysis confirmed that plasmid present in *Shewanella* sp. CGR-L1 is not involved in the γ-HCH biodegradation. *Sphingobium* sp. CGR-L2 was isolated by enrichment method, utilizing γ-HCH as a sole source of carbon. Inoculum size and supplementary glucose concentration were found to be major parameters affecting the rate of biodegradation of

γ-HCH. Under optimal conditions, nearly 87 % of γ-HCH (10 mg l^{-1}) was degraded within 9 hours at 30^0C. γ-HCH at concentrations 25 mg l^{-1} and above retarded the growth of *Sphingobium* sp. CGR-L2, however degradation of γ-HCH was observed up to 250 mg l^{-1} concentration. Degradation assay with technical HCH, confirmed that CGR-L2 degrades γ-HCH, β-HCH and α-HCH isomers. Additionally, a microcosm study was performed which confirmed the degradation of γ- and β–HCH by *Sphingobium* sp. CGR-L2. PCR analysis and sequencing confirmed the presence of genes involved (*lin*A, *lin*B, *lin*C, *lin*D, *lin*E and *linR*) in the biodegradation of HCH in *Sphingobium* sp. CGR-L2. Thus, two γ-HCH degrading bacteria were obtained with different isolation approaches. Our results show that *Sphingobium* sp. CGR-L2 has the potential for use in bioremediation of soils contaminated with HCH.