UGC

Minor Research Project

ON

BIODEGRADATION OF CHLORPYRIFOS BY BACTERIUM ISOLATED FROM PESTICIDE CONTAINING SLUDGE WITH PROCESS CHARACTERIZATION ,OPTIMIZATION AND ITS BIOREMEDIATION AT LAB SCALE

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Summary of the Project

A strain of bacterium capable of highly degrading chlorpyrifos was screened from the soil sample collected from agricultural field. After enrichment, analysis of phenotypic, physiological, biochemical characters and 16SrDNA the organism was identified as Pseudomonas aeruginosa 6A (bc4). Pseudomonas aeruginosa 6A (bc4) strain isolated and characterized from soil sample was used in whole genome sequencing study. Genomic DNA of the selected organism was isolated by CTAB method and characterized by Qubit 2.0 Fluorometer. The paired-end sequencing library was prepared using IlluminaTruSeq Nano DNA HT Library preparation kit. The next generation sequencing for WGS sample were performed using Paired end (PE) 2x150 bp library on Illumina platform. Genomic data revealed presence of total 5649 genes and 149 contigs. The GO (Gene Ontology) annotation classified 3593 genes under different molecular functions, 3804 genes into biological process and 1654 were classified into cellular component category. KEGG Automatic Annotation Server (KAAS) was used to analyze the genes belonging to metabolic pathways. 2516 genes were found to be involved in various metabolic pathways and high percentages of genes were predicted as regulatory genes. The complexity of the 6A (bc4) genome suggest that the bacterium is capable of adaptation into diverse environmental conditions and is able to degrade different pesticides and complex molecules. Analysis of the complete genome sequence of P. aeruginosa reveals many clues regarding the versatility of this organism. It also has broad capabilities to transport, metabolize and grow on organic substances and numerous iron-siderophore uptake systems. Extensions of the work described here have the potential to produce a detailed model for genetic variation in P. aeruginosa with more emphasis on the genes function in bioremediation and pesticide degradation. The effects of carbon source, pH, and temperature and chlorpyrifos concentration on degradation were determined. The biodegradation rates were the highest when the additional carbon source was 0.3%, pH value was 8, chlorpyrifos concentration was 100 mg x L (-1), and cultivated temperature was 30℃. The optimal conditions were proposed, which could provide theoretic basis for prevention and control of pesticides pollution.

Pseudomonas aeruginosa isolate was adapted by subjecting to varying concentrations of chlorpyrifos, i.e. 10, 20, 50, 75 and 100 mg/l in incubator shaker at 37 °C and 150 rpm. An initial 10 mg/l concentration of chlorpyrifos was supplied in minimal salt medium (MSM) under controlled environmental conditions for 3 days. The culture was subsequently scaled up to higher concentrations of chlorpyrifos by transferring one milliliter from the medium with 10mg/L to 25 mg/L of the compound. After every 3 days this process was repeated, each time using medium with higher chlorpyrifos concentration. The entire scale up process continued using enrichment culture technique showed promising capability to utilize chlorpyrifos as a carbon source for their growth. Pseudomonas aeruginosa was adapted to increasing Chlorpyrifos up to 8000ppm. The biodegradation of chlorpyrifos, as assessed by HPLC, showed that chlorpyrifos at 10, 25, 50 mg/l degraded completely over a period of 1, 2 and 3 days, respectively. The intermediate 3, 5, 6 trichloro-2-pyridion, 2, 4-bis (1, 1 dimethyiethyl) phenol and 1, 2 Benzenedicarboxylic acid persisted during bioremediation, but in the long run these converted to CO2, biomass and nutrients. The efficiency of the Pseudomonas aeruginosa isolate as a chlorpyrifos degrader was examined under different culture conditions formulated according to the Plackett-Burman experimental design. A growth medium formulated based on the results of the Plackett-Burman experiment and supplied with 150 mg/L chlorpyrifos recorded 95.12% pesticide decomposition within 48 hr. Inoculation of isolated strain to soil treated 100 mg kg1 chlorpyrifos resulted in a higher degradation rate than in non-inoculated soils. These results highlight the potential of this bacterium to be used in the cleanup of contaminated pesticide waste in the environment. Simultaneously, this isolates along with the pesticide shown good growth of plant Glycine max and comparative result indicates that degradation product of Chlorpyrifos may help the plant growth and an increase in chlorophyll and protein content in PPPs as compared to P, PP, PPs.

CONCLUSION/ OUTCOME OF THE STUDY

Most organophosporus pesticide is harmful to living organism and causes environmental pollution. To overcome this problem we need micro organisms to degrade harmful pesticide from soil. In present work to pseudomonas strains FAP1 and FAP2 were isolated and FAP1 was identified as Pseudomonas aurogenosa 6A (bc4) on the basis of 16 S rDNA , morphological biochemical and whole genome sequencing characterization. The important parameters which significantly increase the % degradation were screen by using Plackett and Burman design. Chlorpyrifos degradation efficiency of pseudomonas aurogenosa was determined by using MSM medium. The degraded components of Chlorpyrifos were detected and identified by HPLC. By pot culture assay, the degraded products resulted in increase in chlorophyll a, b, total chlorophyll and proteins in PPPs as compared to P, PP, and PPs. Similarly, it was estimated that there is an increase in Nitrogen, Phosphorus and Potassium content in PPPs as compared to P. Increased nutrient uptake by plant inoculated with effective bacteria was attributed to the production of plant growth regulator by the bacteria at the root interface, which stimulated root development and resulted in better absorption of water and nutrients from soil. The positive effect of Pseudomonas aurogenosa in this experiments indicated that, bacterial production of plant growth promoting substance might be responsible for observed effect. Isolate can serve as one of the best and cost effective option for removing Chlorpyrifos from contaminated soil.