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Analysis of Plasmid pJP4 Transfer and Its Impact on Bacterial Community Structure in Natural Soil

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The recent developments in biotechnology have led to the construction of genetically engineered microorganisms (GEMs) which can be used for a variety of commercial purposes, including biocontrol and bioremediation. Some of these GEMs will be applied to the open environment, therefore, are apt to escape from research laboratories. Potential hazards resulting from the release of the GEMs into the environment include the survival and persistence of introduced microorganisms and genes, the probability and rate of horizontal gene transfer, and the impact of released GEMs on the microbial community structure in soil.

In this study, we have investigated the transfer of the 2,4-D degradative plasmid pJP4, from an introduced donor strain, *Alcaligenes* sp. JMP228, to the indigenous bacteria and an inoculated recipient, *Burkholderia cepacia* DBO1, in natural soils. The fate of microbes released into the soil was monitored, using their antibiotics resistant properties. In addition, we examined the persistence of *tfdA* gene with competitive PCR, the phylogenetic diversity of transconjugants by 16S rDNA sequence analysis, and the impact of released GEMs on the microbial community structure by denaturing gradient gel electrophoresis (DGGE) of 16S rRNA genes.

Plasmid pJP4 transfer was enhanced in the soils treated with 2,4-D, compared to the soils not amended with 2,4-D. Several different transconjugants were isolated from the soils treated with 2,4-D, while no indigenous transconjugants were obtained from the unamended soils. Inoculation of the soils with both the donor *Alcaligenes* sp. JMP228(pJP4) and a recipient *Burkholderia cepacia* DBO1 produced less diverse transconjugants than the soils inoculated with the donor alone. Repetitive extragenic palindromic-polymerase chain reaction (REP-PCR) analysis of the transconjugants exhibited seven distinct genomic DNA fingerprints. Analysis of 16S rDNA sequences indicated that the transconjugants were related to members of the genera, *Burkholderia* and *Pandoraea*. Denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S rRNA genes revealed that inoculation of the donor caused clear changes in the bacterial community structure of the 2,4-D-

amended soils. The new 16S rRNA gene bands in the DGGE profile corresponded with the 16S rRNA genes of 2,4-D-degrading transconjugants isolated from the soil. The results indicate that introduction of the 2,4-D degradative plasmid as *Alcaligenes* sp. JMP228(pJP4) has a substantial impact on the bacterial community structure in the 2,4-D-amended soil.

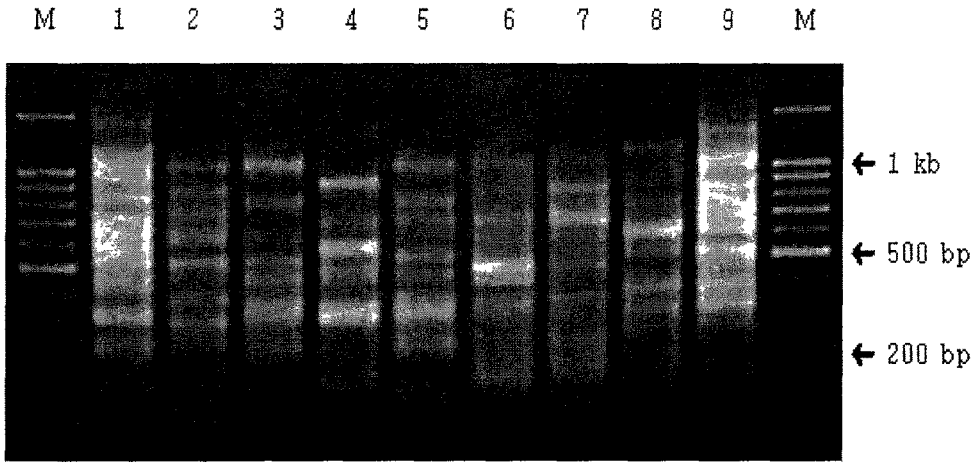

Fig. 1. Genomic DNA fingerprints of the donor, representative transconjugants, and recipient. Lanes: 1, *Alcaligenes* sp. JMP228/pJP4; 2, TC61; 3, TC62; 4, TC81; 5, TC82; 6, TC83; 7, TC84; 8, TC85; 9, *B. cepacia* DBO1/pJP4; M, 100 bp ladder marker.

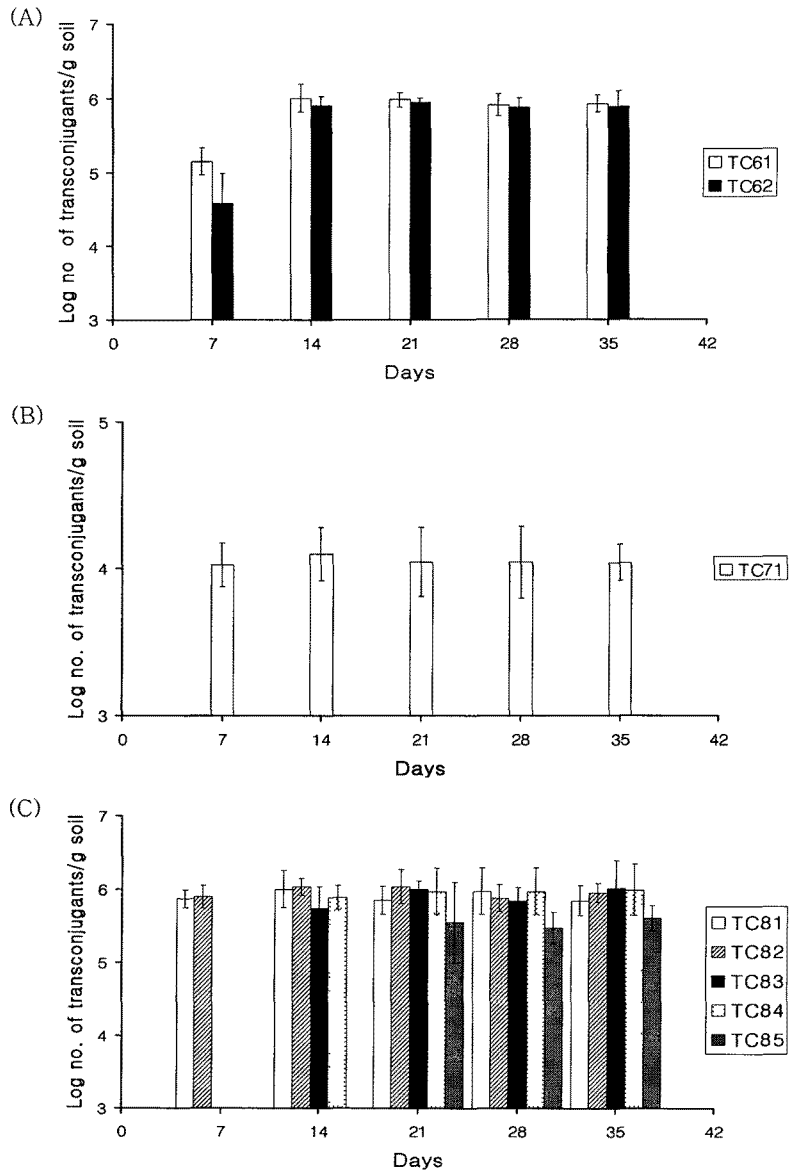

Fig. 2. Occurrence and distribution of the transconjugants in microcosm I (A), microcosm II (B), and microcosm III (C).