

Molecular Analysis of Soil Bacterial Communities Following Application of 2,4-Dichlorophenoxyacetate (2,4-D)

S. Asuming-Brempong^{1*}, S. J. Flynn², and J. M. Tiedje².

¹Soil Science Department, University of Ghana, Legon, Ghana. ²Center for Microbial Ecology and Department of Crop and Soil Sciences, Michigan States University, East Lansing, Michigan 48824

* corresponding author

Abstract

Soil microcosms were constructed from conventional till, zero till and successional field soils to investigate the effect of the management history of the soil on bacterial communities after 2,4-D application. After amended 2,4-D had been degraded, the bacterial community was analyzed by most probable number (MPN) counts of total heterotrophs and 2,4-D degraders and by Small subunit ribosomal DNA (SSU rDNA) analysis using Amplified Ribosomal DNA Restriction Analyses (ARDRA) and Terminal Restriction Fragment Length Polymorphism (TRFLP). The microcosms observed from each of the different field plots showed shifts in the community by both culture and SSU rDNA analyses. The same dominant ARDRA pattern appeared from the SSU rDNA genes amplified from the community DNA from each of the field soils. 2,4-D degrading isolates were also obtained from terminal MPN tubes showing growth on 2,4-D. Some of the isolates also had this ARDRA pattern. Analyses of the partial sequence of the SSU rDNA genes from these isolates identified them as close relatives of the *Burkholderia* genus. Since dominant microbial members selected in each treatment appeared to be the same, the management history of the soil did not influence the selection of dominant 2,4-D degraders. The diversity index measured before and after 2,4-D addition by Shannon-Weiner equation using the ribotype number as species number and peak area the species abundance showed that management history of the soil did influence ribotype diversity.

Key words: Degradation, diversity, herbicide, gene probe, microbial community, ribosomal DNA

Introduction

The use of pesticides such as 2, 4-dichlorophenoxyacetate (2,4-D) continues to increase annually. 2,4-D has been found to be environmentally safe because of the ease by which it is degraded by soil microorganisms (Foster & Mckercher, 1973; Loos *et al.*, 1979). Since the half-life of the herbicide range from 4 to 31 days depending on soil environmental condition and soil type, it is generally accepted that 2,4-D does not persist in the soil beyond one growing season (Smith & Hayden, 1981). However, Thompson *et al.*, (1984) showed that after applying 2,4-D at the normal field rate (1 mg/kg), they observed a chemical residue close to detection limit (approximately 5 µg kg⁻¹) by the end of

the growing season. Even though residues of this magnitude were unlikely to have any biological significance, no studies have been done to elucidate the persistence of high 2,4-D concentrations in sites where the herbicide has been applied for sometime. Microbial community could be different in sites that have a prior 2,4-D history as compared to sites that had not been exposed to the herbicide. Ou (1984) observed higher concentrations of 2,4-D degrading organisms at sites that had been contaminated with 2,4-D compared to sites that had not been exposed to the herbicide. Also, 2,4-D degraders have been isolated from agricultural or Industrial sites exposed to xenobiotic chemicals with success ((Bhat *et al.*, 1994; Chaudry & Huang, 1988; Don &