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DNA- versus RNA-based denaturing gradient gel electrophoresis profiles of a bacterial community during replenishment after soil fumigation

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Abstract

We compared the responsiveness and sensitivity to soil fumigation of DNA- and RNA-based analyses of a bacterial community. We first established an improved RNA extraction method using DNA as an adsorption competitor, because it is extremely difficult to extract nucleic acids from clay-rich volcanic ash soil (Andisol), which adsorbs nucleic acids. This novel method facilitated RNA extraction from 500 mg of Andisol for molecular analyses. Then we monitored 16S rDNA PCR and 16S rRNA RT-PCR denaturing gradient gel electrophoresis (DGGE) profiles of samples collected from a chloropicrin (CP)-treated field over 2 months. The difference between untreated control and CP-treated plots was detected clearly both in DNA- and RNA-based DGGE profiles after treatment. The temporal changes in DGGE profiles, however, differed between DNA- and RNA-based analyses in CP-treated plots. RNA-based DGGE showed quicker and greater changes in the bacterial community after CP treatment than did DNA-based DGGE, which showed similar trends to RNA-based DGGE but with a time lag. The extent of decrease in the diversity index (H') and the change in principal response curves was larger in RNA-based analyses. These results indicate that the rDNA PCR-DGGE method also detects DNA of microbes no longer alive after fumigation, and that rRNA provides a more responsive biomarker than rDNA.

Keywords: Soil fumigation; 16S ribosomal DNA/RNA; PCR-denaturing gradient gel electrophoresis; Principal response curves; RNA extraction from soil; Competitor

1. Introduction

Soil fumigants are used extensively to control plant-parasitic nematodes, weeds, fungi, and insects. Ideally, a pesticide should be toxic only to the target organisms; however, fumigants are a class of pesticide with broad biocidal activity, and they affect many nontarget soil organisms (Ibekwe, 2004). Productivity of agricultural systems is largely dependent on the functions of soil microorganisms (Killham, 1994; O'Donnell et al., 1994), which have important roles, particularly in nutrient cycling and fungistasis (Dobbs and Gash, 1965). Unfortunately, the effects of soil fumigation practices on microbial communities and their recovery following fumigation are largely unknown; only recently has this issue been studied (Ibekwe et al., 2001). Alterations in the microbial community composition may lead to changes that interfere with the functional diversity and, ultimately, the overall soil quality (de Boer et al., 2003).

Molecular techniques used in soil microbial ecology studies have brought new insights into the community structure and dynamics of soil microbes in agricultural fields (Felske et al., 2000; Sessitsch et al., 2001; Smalla et al., 2001; Smit et al., 2001; Ibekwe et al., 2002; Dungan et al., 2003). In particular, the use of PCR to specifically amplify 16S rDNA molecules from DNA extracted directly from a wide range of habitats has allowed the assessment of microbial diversity, including microbial lineages for which there are no known pure cultures. Furthermore, the

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use of denaturing gradient gel electrophoresis (DGGE; Muyzer et al., 1993) to separate mixed PCR products recovered from the environment by specific amplification of bacterial 16S rDNA sequences offers a culture-independent method for tracking dominant bacterial populations across space and time.

DNA directly extracted from soil, however, does not always accurately reflect environmental change. For example, even though some differences were detected in physiological characteristics, numbers of culturable bacteria, and/or microbial biomass, DNA-based bacterial community fingerprints did not show any clear differences between rhizosphere and bulk soil (Duineveld et al., 1998), pesticide-treated and untreated soils (Engelen et al., 1998), heavy-metal-contaminated and uncontaminated soils (Ellis et al., 2003), or soils fumigated with methyl isothiocyanate and untreated soils (Toyota et al., 1999; Kowalchuk et al., 2003). Also, a remarkable similarity was found between DNA-based fingerprints from sterilized and untreated soils (Kowalchuk et al., 2003).

Analyses targeting rRNA, in addition to or instead of rDNA, are expected to be more responsive because, in general, metabolically active cells contain a higher level of intracellular 16S rRNA than do quiescent cells. Several studies succeeded in more responsive detection by using RNA-based approaches (Duineveld et al., 2001; Girvan et al., 2003; Gremion et al., 2003; Nicol et al., 2003; Mahmood et al., 2005; Noll et al., 2005), although some reports showed that RNA-based approaches are subject to the same biases as DNA-based approaches (Norris et al., 2002a; Griffiths et al., 2003a, b).

In addition, technical difficulties remain in extracting nucleic acids from some types of soils. Clay-rich soils (Volossiouk et al., 1995; Frostegård et al., 1999) and volcanic ash soils (Hoshino and Matsumoto, 2004) strongly adsorb DNA and RNA and consequently prevent nucleic acid extraction.

The present study aimed to compare the sensitivity to soil fumigation of DNA- and RNA-based approach. First, we established an improved RNA extraction method using DNA as an adsorption competitor. Second, we monitored and compared DNA- and RNA-based DGGE profiles of the bacterial community during the recovery process after soil chloropicrin (CP) fumigation in an experimental field.

2. Materials and methods

2.1. Field design and sampling

Experiments were performed in a field of the national institute for agro-environmental sciences (NIAES), Tsukuba, Japan (soil characteristics are presented in Table 1). Soil was fumigated with CP in September of 2001, 2002, and 2003 in three replicate plots (each 5 m × 6 m) arranged according to the randomized Latin square method. The fumigant (99.5% CP, Mitsui Chemicals, Inc., Tokyo, Japan) was injected at 17 cm depth with a 30-cm spacing in a hound's-tooth pattern at a rate of 2.4 ml per injection spot (32 ml m^{-2}). The soil was then covered with 0.05-mm-thick polyethylene film for 17 d in both treated and untreated plots. The film was removed, and the soil was tilled to disperse the fumigant. One week later the field was tilled again, magnesium lime (20 kg ha^{-1}) and chemical fertilizer (15.4 kg ha^{-1}, 13N:18P:14 K) were applied, and the field was sown with spinach (*Spinacia oleracea* L. cv. Solomon, Sakata Seed Co., Kanagawa, Japan) with 70 cm between rows. Spinach was cultivated twice a year from October to April and from May to July.

In 2003, samples were taken from three CP-treated plots (plots 3, 5, and 7) and three untreated control plots (plots 2, 4, and 9) 1 d before fumigation (B) and periodically after removal of the film. The day of film removal was designated as 0 d (0D) after treatment, and 0D samples were taken after film removal and before tillage. Additional samples were taken 4 d (4D), 1 week (1W, after spinach sowing), 2 weeks (2W), 1 month (1M), and 2 months (2M) after treatment. Three cores (15 cm of topsoil) were taken from the space between rows in each plot, sieved (2-mm mesh), and mixed to make one composite soil sample. Soil samples were used for plate counts on sampling dates and stored at −80 °C until all the samples were available for molecular analyses.

2.2. DNA extraction from soil

DNA was extracted from 500 mg of soil using the FastDNA Spin Kit for soil (Qbiogene, Inc., Irvine, CA, USA) according to the manufacturer's instructions, except that 40 mg skim milk g^{-1} soil was added to the sodium phosphate buffer. Due to the high DNA adsorption to clay

Table 1
Chemical and physical properties of soils used for RNA extraction

Soil	Origin	Soil taxonomy[a]	Particle distribution (%)				pH (H_2O)	Organic C content (g kg^{-1})	P retention (%)
			Coarse sand	Fine sand	Silt	Clay			
NIAES	Spinach field, Ibaraki	Dystric-Silic Andisol	7.7	18	32.5	41.8	5.46	83.419	83
A	Forest, Hokkaido	Dystric-Haplic Planosol	0.5	14.8	37	47.7	4.55	165.58	43.4
B	Forest, Hokkaido	Dystric-Haplic Cambisol	15.9	32.7	30.6	20.8	4.18	79.22	22.2

[a]According to the world reference base (WRB) for resources classification.