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16S rDNA analysis reveals low microbial diversity in community level physiological profile assays

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Abstract

The metabolic diversity of microbial communities is fundamental for the multiple soil functions mediated by microorganisms. Community level physiological profiles (CLPPs) based on sole C source oxidation have been used as a fast and reproducible tool to study soil microbial functional diversity because the utilisation of available carbon is the key factor governing microbial growth in soil. Our aim was to assess the phylogenetic affiliation of the microorganisms responsible for C consumption after inoculating Biolog™ plates. For this purpose, two semi-arid Mediterranean forest soils with significantly different patterns of C consumption and microbial community structure were used. Following the inoculation of the Biolog plates, suspensions from seven wells were sampled after 1, 2 and 7 d of incubation. DNA was extracted and the microbial communities analysed by polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR–DGGE) and sequencing of excised bands.

Despite major differences in the microbial communities of the soils studied, their DGGE banding patterns after incubation were similar for all the analysed C source suspensions. Microorganisms belonging to β-Proteobacteria (*Ralstonia* sp. and *Burkholderia* sp.) and α-Proteobacteria (*Rhizobium* sp.) were dominant. These opportunists had a competitive advantage under the conditions at which the CLPPs were analysed.

This study reveals that significantly different CLPP patterns can be generated on the basis of only 3–4 genera, as reflected by PCR–DGGE analysis. Also for this reason, CLPPs based on incubations of soil suspensions should just be used as a screening method and always be accompanied by other techniques for community analysis.

Keywords: CLPPs; PCR–DGGE; *Burkholderia* sp.; *Ralstonia* sp.; *Rhizobium* sp., Microbial communities

1. Introduction

Soil microbial communities play a major role in numerous soil functions, such as organic matter turnover, nutrient release, humification, degradation of pollutants, and maintenance of the soil structure (Stevenson, 1982; Preston et al., 2001). A well functioning soil microbial community is a requisite for soil fertility and resilience against disturbing agents.

The assessment of the microbial community structure and composition by molecular techniques provides relatively little information about the microbial functions in soils (Leckie, 2005). Investigating the diversity of microbial heterotrophic functions related to carbon may yield more relevant information about the roles of microorganisms in the ecosystem (Zak et al., 1994), since carbon is a key factor controlling microbial growth in soils (Wardle, 1992). One approach, broadly termed microbial community level physiological profiling (CLPP), was developed based on the ability of soil microbial communities to metabolize a range of organic C substrates (Garland and Mills, 1991). This method has been used as a fast and highly reproducible tool to study soil microbial functional diversity (Kirk et al., 2004), The method proved useful for revealing differences between microbial communities in natural soils (e. g. Zak et al., 1994) soils under different land-use (e. g. Yao et al., 2000; Gomez et al., 2004; Sharma et al., 1998), soils submitted to degradation (e. g. Yan et al., 2000) or treated with herbicides (e. g. El Fantroussi et al., 1999) and other contaminants (e.g. Derry et al., 1998; Konopka et al., 1998). CLPP was among the most sensitive methods to detect effects of elevated CO_2 (Mayr et al., 1999a) and has been used for several other applications. Although the approach for analysing the functional diversity has

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Table 1
Soil properties (In parentheses, standard deviation for $n=3$)

	Pinewood with maquis (PM)	Grassland (GS)
Plant cover	Pinewood with maquis	Grassland
Plant cover (%)[a]	100	27
Slope aspect	North	South
Slope (%)	45	45
Slope position	Backslope	Backslope
Elevation (m)	640	600
Parent material	Colluvial deposits	Limestones
Soil type[b]	Typic Calcixeroll	Typic Haploxerept
Soil texture	Loamy	Loamy
Aggregate stability (%)	35.7	25.0
pH	8.1	8.5
EC (dS m^{-1})	1.42	0.16
TOC (g kg^{-1})	157	20.2
WSC (g kg^{-1})	0.28	0.07
C_{mic} (mg C kg^{-1})	2342	488

[a]In each plot, determined along three 10 m transects placed perpendicular to the slope angle and 5m apart.
[b]Soil Survey Staff (1998).

attracted a lot of attention, largely because data are fairly easily generated and relatively inexpensive, it is essential that its limitations are fully appreciated (Preston-Mafham et al., 2002).

CLPP analysis requires extraction of cells from the soil samples, which are inoculated directly onto commercially available sole-carbon source plates (e.g. Biolog ™). If a C source is degraded, respiratory CO_2 reduces a tetrazolium dye. The production of formazan is directly related to the reducing equivalents formed (Hatzinger et al., 2003) by the inoculated community (Hitzl et al., 1997) and subsequently the growing cells within a well (Haack et al., 1995). Although this is a culture-based assay, it has been found that non-culturable cells respond to substrate supply (Garland and Lehman, 1999). Smalla et al. (1998) note that CLPPs do not necessarily reflect the functional potential of the dominant community members, but are rather biased towards the populations that grow best under the assay conditions.

For this work, we hypothesised that the microbial populations contributing to the CLPPs might be a small part of the soil communities. It was our objective to determine which organisms contribute to the CLPP patterns and how different these populations are among soil samples and C substrates. For this purpose, Biolog™ Ecoplates (Insam, 1997) were incubated with microbial extracts from two soils differing in their physical, chemical and microbiological properties, as well as in their CLPP patterns and microbial community structure. PCR–DGGE and sequencing were used for identification of the microorganisms growing from seven C substrates.

2. Materials and methods

2.1. Soil sampling

Two soil types were sampled in the semi-arid Mediterranean mountain range of Crevillent (Alicante, SE Spain):

PM: North-facing Typic Calcixerolls under an Aleppo pinewood with maquis, dominated by kermes oak (*Quercus coccifera* L.), and with scandent shrubs, grasses and mosses,

GS: South-facing Typic Haploxerepts covered with patchy perennial grasslands dominated by the tussock-forming species *Stipa tenacissima* L.

To sample each soil type, three 100 m^2 plots were randomly established. In each plot, a sample consisting of a mixture of ten homogenised sub-samples was collected in April 2003. Visible plant residues and roots were removed and fresh soil sieved <2 mm and kept at 4 °C. Site characteristics and soil properties are shown in Table 1. Data on microbial community structure, determined by PCR–DGGE, and C consumption patterns in Biolog Ecoplates were presented by Goberna et al. (2005).

2.2. Chemical soil parameters and microbial biomass carbon

Electrical conductivity (EC) and pH were measured in aqueous extracts (1:5 and 1:2.5 w/v, respectively). Total organic carbon (TOC) was determined by oxidation with 1N potassium dichromate in acidic medium and back titration with 0.5 N ammonium ferrous sulphate, as described by Walkley and Black (1934). Water-soluble carbon (WSC) was measured from an aqueous solution (1:5 w/v) using a TOC analyser (TOC-5050A,

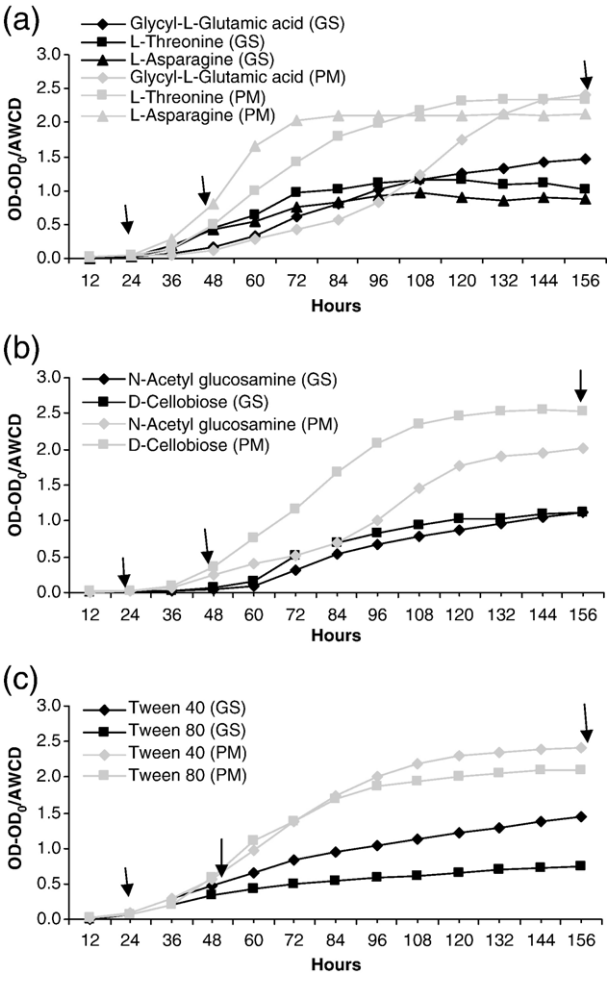

Fig. 1. Normalized data of colour development from different C substrates belonging to different chemical groups (Amino acids (a), Carbohydrates (b) and Polymers (c)) of two different soils (Pinewood with maquis (PM) and Grass steppe (GS)). The arrows show the time of sampling.