Short communication. Response of bacterial community composition to long-term applications of different composts in agricultural soils

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Abstract

Differences in the bacterial community composition of agricultural soils caused by a long-term (12 year) application of different composts were identified by cultivation-dependent and -independent methods (PCR-DGGE and 16S rRNA clone libraries). The number of colony forming units indicated that the successive incorporation of organic amendments increased the bacterial abundance (6.41-5.66 $\log_{10}$ cfu g^{-1} dry soil) compared to control and mineral soils (5.54-3.74 $\log_{10}$ cfu g^{-1} dry soil). Isolated bacteria were dominated by Actinobacteria, whereby compost-amended soils and green compost-amended soils showed, respectively, higher number of members of Actinobacteria (100% and 64%) than control and mineral soils (50% and 40%). The 16S rRNA clone libraries were dominated by Proteobacteria (43%), Acidobacteria (21%) and Actinobacteria (13%). Proteobacteria and Actinobacteria were most abundant in compost amended soils while Acidobacteria were more frequently found in mineral fertilizer and control soils. Partial 16S rRNA gene clone libraries revealed a higher bacterial diversity than cultivation. In conclusion, we found differences of bacterial community composition with a cultivation approach and clone libraries between compost amended soils and control and mineral soil.

Additional key words: 16S rRNA clone libraries; fertilizer; isolated bacteria; organic amendments.

Resumen

Comunicación corta. Respuesta de la composición bacteriana de un suelo agrícola a la incorporación a largo plazo de diferentes composts

Se estudiaron las comunidades bacterianas de suelos agrícolas enmendados durante 12 años con diferentes tipos de compost, mediante el empleo de técnicas de cultivo en placa y técnicas moleculares (PCR-DGGE y genotecas de ARNr 16S). El cultivo en placa mostró un aumento del número de bacterias en los suelos enmendado con diferentes composts (6,41-5,66 $\log_{10}$ cfu g^{-1} suelo), respecto a los suelos no enmendados o con fertilización mineral (5,54-3,74 $\log_{10}$ cfu g^{-1} suelo). Esta técnica reveló que las Actinobacterias componen la mayoría de las bacterias identificadas en placa, encontrando un mayor número en los suelos enmendados con diferentes compost (100%-64%) frente a los no enmendados o con fertilización mineral (50%-40%). Las genotecas permitieron identificar además de Actinobacterias (13%), la presencia de Proteobacterias (43%) y Acidobacterias (21%). En los suelos enmendados con compost predominaron Proteobacterias y Actinobacterias, mientras que en los suelos sin enmendar y con fertilización mineral predominaron las Acidobacterias. Las genotecas mostraron mayor diversidad bacteriana que el método de cultivo en placa. En conclusión, los datos obtenidos mediante cultivo en placa y con genotecas mostraron diferencias en la composición de la comunidad microbiana entre suelos enmendados con compost y aquellos sin enmendar y con fertilización mineral.

Palabras clave adicionales: bacterias aisladas; enmiendas orgánicas; fertilizantes; genotecas bacterianas.

Soil application of compost made from organic wastes is gaining importance since integrated and biological agriculture is becoming increasingly popular (Lalande *et al.*, 2000; Masciandaro *et al.*, 2000). Compost is considered to be an environmentally safe, agronomically advantageous, and relatively cheap organic amendment able to stimulate soil microbial activity, improve crop growth (Pascual *et al.*, 1997), avoid organic matter loss (Ros *et al.,* 2003) and increase microbial diversity (Peacock *et al.*, 2001). Composition and activity of soil bacterial communities are key issues due to their fundamental role in biogeochemical cycles and in the formation of soil structure (Lynch *et al.*, 2004). Bacterial communities can be measured by various techniques, *e.g.* traditional plate counting and molecular based-techniques such as PCR-based approaches. However, each one of them has their particular limitations. For this reason, the best way to study soil bacterial communities is to use a variety of different approaches to obtain the broadest picture possible.

Few studies have focused on the response of bacterial communities to long-term compost applications to agricultural soils (*e.g.* Saison *et al.,* 2006), some of them have been carried out on basis of the field experiment that is the basis of the present study, involving PCR-DGGE, phospholipid fatty acid fingerprinting, community level physiological profiling and volatile organic compound profiling (Innerebner *et al.,* 2006; Ros *et al.,* 2006a, b; Seewald *et al.,* 2009). The objective of this work was to give additional evidence for compost effects on bacterial community composition in a long-term crop rotation experiment with continuous compost amendments using a cultivation approach and clone libraries.

From 1991 on, a long-term crop rotation [maize (*Zea mays* L.), summer-wheat (*Triticum aestivum* L.) and winter-barley (*Hordeum vulgare* L.)] field experiment was performed near Linz (Austria). The soil was loamy silt (17.4% clay, 69% silt, 13.6% sand) with a pH (H_2O) of 6.8. The soil contained 1.9% organic matter, and 260 and 300 mg kg^{-1} available P and K, respectively. The experiment was performed using twelve randomly distributed plots (4 treatments with 3 replicates; 10×3 m). Treatments were performed annually in spring time as follows: a) Control, soil without fertilization; b) OWC, annual application of compost from source-separate collection of domestic organic waste corresponding to 175 kg N ha^{-1} ($12.6 \cdot 10^3$ kg compost ha^{-1}) plus 80 kg N (NH_4NO_3) ha^{-1}; c) GC, annual application of green waste compost from roadside and park leaves corresponding

Table 1. Average chemical properties of the different composts. Data on a dry mass basis and an average of chemical properties obtained during 12 years for each compost. Numbers in parenthesis indicate standard deviation ($n = 12$)

Chemical properties	Urban organic wastes compost (OWC)	Green compost (GC)
Organic matter (g kg^{-1})	330 (42)	350 (29)
Total nitrogen (g kg^{-1})	12 (1.8)	16 (0.6)
C/N	16 (2.0)	13 (1.2)
P$_2$O$_5$ (g kg^{-1})	27 (1.3)	14 (1.0)
K$_2$O$_5$ (g kg^{-1})	6 (0.5)	13 (0.5)
Cu (mg kg^{-1})	70 (1.5)	38 (1.3)
Zn (mg kg^{-1})	277 (4.5)	163 (3.8)
Ni (mg kg^{-1})	21 (1.3)	22 (1.0)
Cr (mg kg^{-1})	32 (1.2)	28 (0.6)
Pb (mg kg^{-1})	75 (2.8)	28 (2.2)
Cd (mg kg^{-1})	0.56 (0.02)	0.34 (0.01)
Hg (mg kg^{-1})	0.24 (0.01)	0.15 (0.009)

to 175 kg N ha^{-1} ($9.3 \cdot 10^3$ kg compost ha^{-1}) plus 80 kg N (NH_4NO_3) ha^{-1}; d) Mineral: mineral fertilization treatment corresponding to 80 kg N (NH_4NO_3) ha^{-1}. The main characteristics of composts are given in Table 1. Soils were sampled on October 15, 2003 after maize harvest. Ten random soil cores (6 cm diameter, depth 0-20 cm) from each treated soil were taken. These samples were pooled to reduce spatial heterogeneity and sieved (< 2 mm).

Isolation of bacterial cultures was performed from the higher dilutions of each sample; the most frequently occurring bacteria were selected, and picked according to morphological characteristics such as colour, shape and type growth. They were subcultured to assume that they were pure and preserved on a Standard I agar as described by Mayrhofer *et al*. (2006), but omitting cycloheximide. After 7 d at 25°C, colony forming units (CFUs) of each sample were counted and DNA from the isolated bacteria was extracted using the GenElute Bacterial Genomic DNA kit (Sigma). Bacterial DNA was amplified with primers 338f-GC/907r as described in Ros *et al*. (2006b). Microbial community DNA was extracted from the bulk soils (0.4 g) using the Fast DNA Spin Kit for soil (BIO 101, USA). The extracted DNA was subjected to PCR amplification with the 63f/1378r primer set, to generate nearly full-length 16S rRNA clones (Marchesi *et al.,* 1998). Three independent PCR reactions from each soil sample were mixed and submitted to cloning with the pCR® 4-TOPO® TA cloning® kit for sequencing (Invitrogen™ life technologies). The presence of inserts was determi-

ned by direct PCR with the primer set 338f-GC/907r. Clones and pure cultures were screened using DGGE (Ros *et al.,* 2006b). DNA from bacterial cultures and clones were reamplified and sequenced (Microsynth GmbH, Balgach, Switzerland). Sequences considered chimeras by CHIMERA_CHECK (Cole *et al.,* 2005) were excluded. The rRNA gene sequences (532 bp) were submitted to a GenBank database under accession numbers GU946084 to GU946211. Sorensen's similarity coefficient (S_c) based on species presence *vs.* absence was calculated using the following function: $S_c = 2c/(a + b)$, where a = number of species present in sample 1, b = number of species present in sample 2, and c = number of species shared by both samples.

The number of CFUs ($\log_{10}$ cfu g^{-1} dry soil) was significantly higher ($p \leq 0.05$) in soils treated with OWC (6.41 ± 0.06), GC (5.66 ± 0.05), or mineral fertilizer (5.54 ± 0.03) as compared to control soil (3.74 ± 0.06) due to the incorporation of organic matter and nutrients that activate the autochthonous microorganisms of the soil (Pascual *et al.,* 1997). The highest number of CFUs in OWC may be attributed to the easy degradability of its organic matter compared to the recalcitrant material of the green waste compost (more bulky cellulose and lignin-rich materials) (Pascual *et*

al., 2000). This increase in CFUs supports earlier data on positive effects of compost amendments on microbial biomass (Ros *et al.,* 2006a) probably due to the increased availability of substrate C that stimulates microbial growth, but a direct effect from microorganisms added with the compost is also possible (García-Gil *et al.,* 2000; Ros *et al*., 2003).

A total of 24 different isolates were selected based on their colony morphology. Sequences were distributed among three phyla, Actinobacteria (62.5%), Firmicutes (25.0%) and Proteobacteria (12.5%). GC and OWC isolates mainly belonged to the phylum Actinobacteria (100% and 63.7% respectively), being assigned to the genera *Microbacterium, Arthrobacter, Streptomyces, Mycobacterium, Rhodococcus* and *Micromonospora* (Table 2). In control and mineral fertilizer soils the distribution of Actinobacteria, Firmicutes (*Bacillus, Staphylococcus*) and γ-Proteobacteria (*Pseudomonas* and *Lysobacter)* was balanced (Table 2). The low diversity of cultivated bacteria may be attributed to the simple cultivation approach chosen and a short incubation time. Most of the isolates were identified as members of the phylum Actinobacteria that includes some of the most common soil microorganisms, playing important roles in decomposition and humus formation (Felske

Table 2. Phylogenetic assignments of control, mineral, organic waste compost (OWC) and green compost (GC) amended soil isolates

Phylogenetic affiliation	Number of isolates				Closest phylogenetic relative (accession number)	Query coverage (%)
	Control	**Mineral**	**OWC**	**GC**		
Actinobacteria						
Microbacterium		1	2		*M. trichothecenolyticum (EF204433)*	100
Arthrobacter		1	3		*Arthrobacter sp.(DQ985282)*	100
Streptomyces			1	4	*Streptomyces sp.(AF131506)*	
					Streptomyces sp.(AF131549)	100
Rhodococcus			1		*Rhodococcus sp.(AJ306173)*	100
Mycobacterium	1				*Mycobacterium sp.(AF480587)*	100
Micromonospora	1				*Micromonospora sp.(DQ994710)*	100
Firmicutes						
Bacillus	1	1	3		*Bacillus sp.(EU648140)(FN293225)*	100
Staphylococus		1			*Staphylococcus sp.(AB192374)*	100
Proteobacteria						
γ-Proteobacteria		1			*Rahnella sp.(DQ836258)*	100
Pseudomonas	1				*Pseudomonas sp.(FJ596393)*	100
Lysobacter			1		*Lysobacter sp.(DQ530133)*	100
Total (all taxa)	4	5	11	4		

et al., 1997; Rheims *et al*., 1999). Due to the difficult lysis of Actinobacteria cells, DNA extraction from members of this phylum is hampered (Feinstein *et al*., 2009), which is one possible explanation why Actinobacteria typically only display 10-16% in clone libraries from soil ecosystems, while they represent 40-100% of cultured soil bacteria (Kaiser *et al.,* 2001).

A total of 122 clones from 200 were selected from the clone libraries of the different treatments. Clones were affiliated to nine phyla generally found in soils (Janssen, 2006), the predominant being Proteobacteria (43%), Acidobacteria (21%) and Actinobacteria (13%). Members of the phyla Firmicutes, Bacteroidetes, Gemmatimonadetes, Chloroflexi, Nitrospirae and Cyanobacteria as well as unclassified bacteria were also found (Fig. 1). Proteobacteria encompass an enormous morphological, physiological and metabolic diversity, and are of great importance to global carbon, nitrogen and sulfur cycling (Kersters *et al*., 2006). The percentage of Proteobacteria was higher in OWC and GC (50 and 48% respectively) than in the control and mineral fertilizer treatments (40 and 32% respectively). In the OWC and GC treatments α-Proteobacteria were predominant, while in the control and mineral fertilizer treatments γ-Proteobacteria dominated. The percentage of Actinobacteria members was higher in OWC (20%) and GC (13%) than in mineral and control soil (10%). However, the percentage of sequences belonging to Acidobacteria was higher in control and mineral soil (27 and 26%, respectively) than in OWC and GC amended soil (17 and 16%) (Fig. 1). Similar results were observed by other authors (*e.g.* Sessitsch *et al.*, 2001; Valinsky *et al*., 2002; Sun *et al*., 2004; Toyota and Kuninaga, 2006) where variations in the composition

of bacterial community also depended on the soil type, sample origin and plant growth stage.

Smit *et al*. (2001) suggested that the ratio between Proteobacteria and Acidobacteria frequency can be indicative of the soil nutrient status when calculated as % Proteobacteria/(sum of % Proteobacteria and % Acidobacteria). We found a higher ratio for OWC (0.82) and GC (0.75) compared to control (0.60) and mineral soil (0.64). This indicated a higher nutrient supply in the compost amended samples (Ros *et al*., 2006a). In comparison, a ratio of 0.87 was observed by McCaig *et al*. (1999) in high-input agricultural soil.

The Sorensen's similarity coefficients among treatments were very low; the highest similarity was observed between GC and OWC (0.14). It could indicate that the incorporation of both organic amendments changed the microbial community composition in a similar way. This was supporting earlier findings by Innerebner *et al*. (2006) and Ros *et al*. (2006a), who found a greater microbial diversity in the compost amended samples than in the control and mineral fertilizer samples. A very low species overlap ranged (0.09-0.00) was detected for the rest of overlapping between the treatments.

A Sorensen's similarity coefficient of 0.15 reflects the low overlap between taxa detected by cloning and isolation techniques. This supports earlier findings by Suzuki *et al.* (1997) and Kaiser *et al.* (2001). In contrast, Chandler *et al*. (1997) showed an overlap of 41%. The amount of overlap between cultured organisms and clone libraries depends on several factors, such as the complexity of the environment, the discrepancy between plate counts and direct counts, the media and culture conditions and the sample size of 16S rRNA clones (Dunbar *et al.,* 1999). However, despite the biases
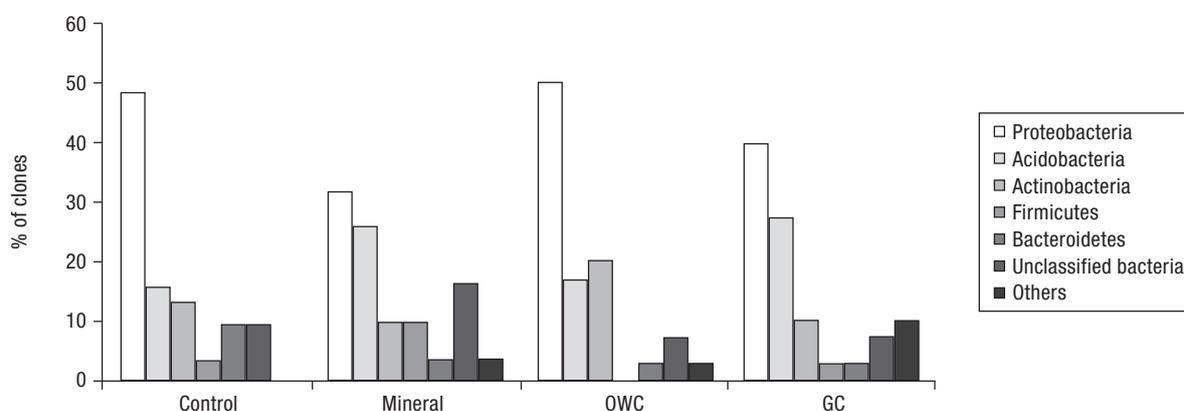

Figure 1. Phylogenetic distribution identified in 16S rRNA clone libraries of different treatments. Total clones: control (30), mineral (31), organic waste compost (OWC, 30) and green compost (GC, 31).

inherent in each method, both methods also identified compost amended soils to be similar in terms of phylotype composition compared to control and mineral soils.

In conclusion, we found differences of bacterial community composition with a cultivation approach and clone libraries between compost amended soils and control and mineral soil. Compost amended soils are dominated by Proteobacteria and Actinobacteria and control and mineral soil by Acidobacteria.

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