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Role of vermicompost chemical composition, microbial functional diversity, and fungal community structure in their microbial respiratory response to three pesticides

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ABSTRACT

The relationships between vermicompost chemical features, enzyme activities, community-level physiological profiles (CLPPs), fungal community structures, and its microbial respiratory response to pesticides were investigated. Fungal community structure of vermicomposts produced from damaged tomato fruits (DT), winery wastes (WW), olive-mill waste and biosolids (OB), and cattle manure (CM) were determined by denaturing gradient gel electrophoresis of 18S rDNA. MicroResp™ was used for assessing vermicompost CLPPs and testing the microbial response to metalaxyl, imidacloprid, and diuron. Vermicompost enzyme activities and CLPPs indicated that WW, OB, and DT had higher microbial functional diversity than CM. The microbiota of the former tolerated all three pesticides whereas microbial respiration in CM was negatively affected by metalaxyl and imidacloprid. The response of vermicompost microbiota to the fungicide metalaxyl was correlated to its fungal community structure. The results suggest that vermicomposts with higher microbial functional diversity can be useful for the management of pesticide pollution in agriculture.

1. Introduction

Vermicomposting is a low-cost biotechnology which enables the recycling of a variety of wastes from different nature through the combined action of earthworms and microorganisms. Vermicomposts were early reported as bioactive amendments housing microbial communities relevant to improve soil fertility (Kale et al., 1992). Apart from being excellent organic products for agriculture, vermicomposts can be considered useful materials for restoring pesticide contaminated soils as they enhance the adsorption of pesticides reducing the environmental risk of pesticide leaching towards groundwaters (Romero et al., 2006). Fernández-Bayo et al. (2009) reported that amending soils with vermicomposts fosters dissipation of pesticides, such as diuron in agricultural soils. On the other hand, vermicomposts have also been reported as suitable materials for developing bioremediation tools such as biobarriers (Moreno et al., 2009) or biocovers (Moon et al., 2010). In this sense, vermicomposts could be promising organic materials to constitute biomix layers in biobed systems, like thermophilic-composts, which have already been reported to be effective for adsorbing and degrading pesticides in biobeds (Vischetti et al., 2008). Recently, Blaszak et al. (2011) have isolated microorganisms capable of biodegrading the pesticide simazine from a vermicompost produced from manure, suggesting that vermicomposts are a source of pesticide-biodegrading microorganisms. Hence, further knowledge on the resident microbial community in a vermicompost could help to predict its potential utility for pesticide bioremediation.

It is expected that vermicomposts produced from different parental wastes have different chemical compositions and dissimilar autochthonous microbial communities. However, to date, the interrelationships between the chemical features of different vermicomposts and the functional diversity of their resident microbiota are still unclear. Moreover, there are no studies which provide information on the connection between the functional diversity of vermicompost microbiota and its responses to pesticides. Thus, it remains to be elucidated to what extent microbial functional diversity of a vermicompost could be related with its potential utility for pesticide bioremediation. Relevant information on this topic could be inferred by investigating the impact of pesticides on the microbiota of different vermicompost types along with their chemical features and microbial functional diversity. The microbial functional diversity of different vermicomposts can be assessed by

Abbreviations: CLPP, community-level physiological profile; DGGE, denaturing gradient gel electrophoresis; PCA, principal component analysis; RI, respiration index; TOC, total organic carbon; DT, vermicompost from damaged tomato fruits; WW, vermicompost from winery wastes; OB, vermicompost from olive-mill waste mixed with biosolids; CM, vermicompost from cattle manure; WSC, water soluble carbon.

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determining their community-level physiological profile (CLPP), which captures the ability of the vermicompost microbiota to metabolize single carbon substrates (Campbell et al., 2003; Mondini and Insam, 2005). Hill et al. (2000) reported that if CLPPs from two samples are clearly separated, then the functional diversity of their resident microbial communities can be considered different. MicroResp™ is a micro-respiration system designed for determining the CLPPs of microbial communities housed in whole-substrates, avoiding the disadvantages of other CLPP approaches based on culturing of microorganisms on plates such as the Biolog system (Campbell et al., 2003). In addition, the great versatility of this system makes it useful for assessing the effect polluting substances, such as pesticides, on the respiratory activity of microbiota inhabiting organic substrates (Campbell et al., 2003). On the other hand, the assessment of enzyme activities, such as oxidoreductases and hydrolases, in vermicomposts has also been reported useful for studying the biochemical functional diversity of vermicompost microbiota (Benítez et al., 1999; Vivas et al., 2009). Recently, Sen and Chandra (2009) reported that the changes in enzyme activities and CLPP occurred during the vermicomposting of sugarcane waste allows assessing the functional diversity of the microbiota involved in vermicomposting. Hence, the joint analysis of enzyme activities and CLPPs seems to be a suitable approach to investigate the functional diversity of microbial communities housed in diverse vermicomposts. On the other hand, the high activity of some hydrolytic enzymes in a vermicompost could be related to the potential ability of its resident microbiota to degrade certain organic substrates and xenobiotics. For instance, Fernández-Bayo et al. (2009) suggested that the high urease activity found in diuron contaminated soils, which had been amended with a vermicompost produced from spent grape marc, could be related to the hydrolysis of this nitrogen-containing herbicide as ureases catalyses the cleavage of N–C bonds in ureic compounds.

Fungal communities play a very important role as an active component of the vermicompost microbiota (Aira et al., 2006). Previous studies reported that vermicomposts house a high diversity of fungi able to degrade a variety of compounds (Anastasi et al., 2005). In addition, most fungi tolerate high concentrations of polluting chemicals, since they have a complex enzymatic machinery able to degrade complex polymers and xenobiotics such as pesticides (Bending et al., 2002). Thereby, the fungal community structure in vermicompost might be related to the response of its microbiota to pesticides. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 18S rRNA gene fragments has been reported as a useful fingerprinting technique for determining the structure of fungal communities inhabiting complex environmental samples (Vainio and Hantula, 2000). Previous studies have highlighted that DGGE is an easy, fast and reproducible technique to compare the fungal community development during the different phases of vermicomposting (Fernández-Gómez et al., 2010).

In view of the above, this study investigated the chemical features, enzyme activities (dehydrogenase, β-glucosidase, acid phosphatase, and urease), community-level physiological profile, and the fungal community structure of four different vermicomposts, exploring the relationships between these parameters and the respiratory response of the vermicomposts' microbiota to the fungicide metalaxyl, the insecticide imidacloprid, and the herbicide diuron.

2. Methods

2.1. Vermicompost collection

The vermicomposts analysed in this study were the following: a vermicompost from damaged tomato fruits (DT), one from winery wastes (WW), one from olive-mill waste mixed with biosolids (OB), and one from cattle manure (CM). All these vermicomposts were produced by using the earthworm species *Eisenia fetida*. DT was produced by vermicomposting damaged tomato fruits through an indoor continuous-flow reactor as described by Fernández-Gómez et al. (2010). Briefly, 500 g of earthworms were inoculated in a layer of sheep manure (15 kg) placed in the bottom of the reactor, and 10 kg of damaged tomatoes were applied on that layer every week for five months. Afterwards, the earthworms were removed by hand and the organic substrate was left maturing in the reactor for two months without further waste addition. WW was produced by vermicomposting of spent grape marc mixed with lees cake at a ratio 1:1 (dw:dw) by adding an earthworm biomass equivalent to 10% of the waste mass (dw) contained in the bed. Waste moisture was kept at 80–85% by periodical watering during the vermicomposting process. After six months, the earthworms were removed by hand and the resulting organic substrate was finally matured for two months without further water addition. OB was obtained from wet olive cake mixed with municipal biosolids at a ratio 8:1 (dw:dw) after six months of a vermicomposting process which was similar to that of WW. CM was commercially produced by Lumbricor S.L. (Córdoba, Spain) from cattle manure, which was vermicomposted on a large-scale windrow system for four months plus one month of maturation. All these vermicomposts were homogenised and three samples of 250 g were separately taken and ground (<2 mm).

2.2. Chemical analyses

Vermicompost pH and electrical conductivity (EC) were measured with a glass electrode using a 1:10 sample:water (dw:v) ratio. Total organic carbon (TOC) and total nitrogen (N) were determined with a LECO TruSpec CN analyzer (LECO Corporation, St. Joseph, USA). Water soluble carbon (WSC) was extracted by mechanical shaking at 60 °C for 1 h with distilled water (1:10 sample:water; dw:v). Humic acid like (HAL) and fulvic acid like (FAL) compounds were extracted from 2 g of sample by mechanical shaking at 37 °C for 2 h with 40 ml of a solution consisting of 0.1 M $Na_2P_4O_7$ and 0.1 M NaOH. This extract was subsequently acidified to pH ≈ 1 with H_2SO_4 and centrifuged at 3500 rpm to separate the HAL fraction that precipitated from the FAL fraction, which remained in solution. The HAL solution was then obtained by dissolving the precipitate in 10 ml of 0.5 M NaOH. The C content in the WSC, HAL, and FAL solutions was determined by dichromate oxidation followed by titration with ferrous ammonium sulphate.

2.3. Enzyme activity analyses

Dehydrogenase activity was determined using iodonitrotetrazolium formazan (INTF) as substrate, as described by García et al. (1997). β-glucosidase and acid phosphatase activities were analysed by determining the amount of p-nitrophenol (PNP) produced from 4-nitrophenyl-β-D glucanopyranoside (PNG) and 4-nitrophenyl phosphate (PNPP) as described by Tabatabai (1982), and Tabatabai and Bremner (1969), respectively. Urease activity was determined using urea as a substrate as described by Kandeler and Gerber (1988). Each enzyme activity was determined per triplicate using 0.2 g of vermicompost sample.

2.4. Microresp™ analysis

The community level physiological profiles (CLPPs) were determined by using the micro-respiration system Microresp™ with eleven carbon sources: five carbohydrates (L-arabinose, D-xylose, N-acetyl-D-glucosamine, D-trehalose, D-raffinose), four amino-acids (L-arginine, L-cysteine, D-lysine, glycine), and two organic acids (DL-malic acid and D-galacturonic acid), which are ecologically relevant