In vitro elucidation of suppression effects of composts to soil borne pathogen *Phytophthora nicotianae* on pepper plants using 16S amplicon sequencing and metaproteomics.

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ABSTRACT

Compost production is a critical component of organic waste management, and one of its important properties is their suppressivity against soil borne pathogens such as *Phytophthora nicotianae* on pepper plants. Both physico-chemical and biological properties of composts can be responsible of the suppression of pathogens, considering that biological properties are the main driver.

Composts with various levels of suppressiveness against *Phytophthora nicotianae* were analyzed. Physico-chemical properties as pH and Electrical conductivity, and biological properties as microbial activity, amplicon sequencing and metaproteomics were analyzed, considering that the linkage between community structures and proteins may provide deep insights into the mechanism of compost suppressiveness.

Our results indicated that there are differences between suppressive and non-suppressive composts at the phylogenetic level (sequencing) and at the functional level (analysis of Cluster of Orthologous Groups, COGs). Proteins identified were assigned to carbohydrate process, cell wall structure and inorganic ion transport and metabolism. Proteobacterias could be also new indicators of suppression of *Phytophthora nicotianae.*

Keywords: Metaproteomics, compost, sequencing, *Phytophthora nicotianae,* suppressive

Introduction

Composting is a microbial aerobic decomposition, under controlled conditions, that allow the generation of high temperatures by thermophilic microbes. The end product is stable and free of pathogens and viable weed seeds thus ready to be used in plant culture (Martin and Brathwaite, 2012). The type of feedstock, the compost formulation, the composting process, system and management have all been reported to affect compost maturity and quality. They can be used as organic amendment or organic substrate (Haug, 1993; Rynk, 1992; Sullivan and Miller, 2000). One of the particular properties of compost is their suppressive ability against pathogens, that it has been widely linked to living microorganisms (Bonanomi et al., 2010). Studies have shown that heating or autoclaving eliminates the compost suppressive capacity and this capacity can be recovered by mixing these heated or sterilized composts with natural composts (Hoitink and Fahy, 1986). These results point to a suppressive capacity of composts mediated by their microbial community. In addition, physico-chemical properties such as nitrogen, pH, C/N ratio, heat, moisture or degree of compost maturity have been also suggested to be associated with suppression activity (Hoitink and Grebus, 1997). Several studies have focused on the use of different biological parameters (e.g. population, diversity, activity and function of microbes) in compost to develop disease-suppressive composts. Bonanomi et al. (2010) concluded after an extensive data review that fluorescein diacetate hydrolysis, basal respiration, microbial biomass, total cultivable bacteria, fluorescent *Pseudomonas* and *Trichoderma* populations provide the best predictions of disease suppression.

The development of high-throughput molecular tools allows characterizing the taxonomic, phylogenetic, and functional diversity of soil/composts microbial communities to find new predictors of the suppressive capacity of composts. Sequencing has been used to explore structure and composition of composts microbial communities (de Gannes et al., 2013; Neher et al., 2013) and composts suppressive capabilities (Yu et al., 2015; Blaya et al., 2016). However, genomic analysis reveals the total abundance of microbial populations but not the active populations (Bastida et al., 2016). Microbial populations can be in sporulated and dormancy forms. Moreover, it has been recently discovered that up to 50% of the microbial nucleic acid sequences in environmental samples can correspond to death biomass, non-active (Carini et al.,

2016). "Meta-proteomics" was defined by Wilmes and Bond (2006) as the large-scale characterization of the entire protein complement of environmental microbiota at a given point in time. Proteins which have been synthesized by microorganism at the time of sampling, reflect the actual functionality with respect to metabolic reactions and regulatory cascades, and give more direct information about microbial functionality than genes (Willmes and Bond, 2006). By now, microbial proteins have been extracted from different environmental matrices such soil samples (Chourey et al., 2010; Bastida et al., 2014, 2015), leaf litter (Schneider et al., 2012) and compost samples (Liu et al., 2015) and identified with accurate mass spectrometer (MS/MS) equipment and databases that allowed a functional and phylogenetic classification of the identified proteins.

Our study extents the knowledge about the microbial key-players involved in compost suppression capacity. This study was designed to test the hypothesis that the microbial communities found in suppressive compost are taxonomically and functionally distinct compared with those found in non-suppressive composts, so we could find more accurate bioindicators able to predict the suppressive capacity of composts.

Materials and Methods

Composts composition and characterization

Three composts derived by a mix of different by-products, sludge from fruit and vegetable processing industries in the Region of Murcia, and pruning wastes as bulking agent were used. The composts are: **C1**: pepper sewage sludge (25%), garlic residue (25%), almond shell powder (25%), and pruning wastes (25%); **C2**: orange residues (22%), pepper residues (11%) artichoke residues (28%), and pruning wastes (39%); **C3**: pepper sewage sludge (24%), garlic residues (3%), carrots residues (36%), pepper residues (3%), almond shells powder (5%), and pruning wastes (29%).

Composting process was developed according to Morales et al. (2017) briefly here described. The composting processes remained between 100 and 120 days, between 45 and 55 days for the bio-oxidative phase, and when the temperature was constant the maturation phase started with a duration around 2 months. The piles were watered to maintain moisture content of 40% and turned when piles reached

temperatures above 65 ºC. Physico-chemical, chemical and biological characteristics of the composts are shown in Table 1. The pH and electrical conductivity (EC) of the composts were measured in a 1:10 (w/v) water-soluble extract, pH meter and conductivity meter (Crison), respectively. The total organic carbon and nitrogen were measured with an Elemental Analyzer (LECO TruSpec C/N) and nutrients by ICP-OES (ICAP 6500 DUO). The method described by García et al. (1997) was used to measure dehydrogenase activity, reducing 2-p-iodophenyl-3-p-nitro-phenyl-5-phenyltetrazolium chloride (INT) to iodonitrophenyl-formazan (INTF), which was measured in a spectrophotometer at 490 nm.

Disease suppression potential assay

An experiment to test the capability of these composts to suppress *Phytophthora nicotianae* (*P. nicotianae*) was carried out. Each compost was mixed with peat (1/1; v/v) ratio (C1, C2 and C3). Peat itself was used as a control (peat) (Table 1). Seeds of pepper (*Capsicum annuum* cv. Lamuyo) were sown in trays of 150 pots, with one seed per pot and a covering of vermiculite. Eight replicates per compost were established randomly, each replicate consisting of 10 seeds. Germination was carried out in a germination chamber at 28±1 ºC and 70-75% relative humidity. Once seeds germinated, trays were located in a growth chamber (25±1 ºC day (16h); 23±1 ºC night (8h); 70-75% relative humidity). Six replicates of each compost were inoculated with 2 mL of *P. nicotianae* (5 10^3 cfu g^{-1} substrate) after the first true leaf appeared (inoculated) and the other two replicates were not inoculated (non-inoculated). The plant pathogen inoculum was prepared following the method described by Blaya et al. (2016). The suppressive effect of the different composts was determined by measuring the number of dead plants 23 days after inoculation.

Composts were storage at 4˚C until *in vivo* experiment. Composts samples were kept at -20˚C for further genomic and metaproteomic analyses.

DNA extraction, PCR and sequencing

Total DNA was extracted from composts (0.5g) using the Dneasy PowerSoil Kit (Qiagen, Germany) and purified with a QIAquick Gel extraction kit (Qiagen) following

manufacturer's instructions. DNA was quantified using the Quant-iT™ PicoGreen ds DNA kit (Invitrogen) and checked for quality on an agarose gel.

PCR amplification of the V4 region of bacterial 16S rRNA was amplified using barcoded primers 515F and 806R (Argonne National Laboratory), (Caporaso et al., 2012), while fungal ITS2 region was amplified using barcoded primers gITS7 and ITS4 (Ihrmark et al., 2012) in three reactions per sample. Each 25 µl PCR mix contained 2.5 µL of 10x buffer for DyNAzyme DNA Polymerase, 0.75 µL of BSA (20 mg ml^{-1}), 1 µL of each primers (0.01 mM), 0.5 µL of PCR Nucltoide Mix (10 M each), 0.75 µL polymerase (2 U uk-1 DYNAZyme II DNA polymerase 1:24 Pfu PDNA polymerase) and 1 µL of template DNA. Cycling conditions were 94 ºC from 5 min, 35 cycles of 94 ºC form 1 min, 62 ºC for 1 min, and 72 ºC for 1 min, and a final extension at 72 ºC for 10 min. PCR products were purified using the MinElute PCR Purification kit (QIAgen) according to manufacturer's instructions. TruSeq PCR-Free kit (Illumina) was used for library preparation. Sequencing of fungal and bacterial amplicons was performed on Illumina MiSeq C4SYS facility, Institute of Microbiology of the CAS, Prague, Czech Republic.

Sequence data processing and analysis

The amplicon sequencing data were processed using the pipeline SEED 1.2.31 (Větrovský and Baldrian 2013) as described by Zifcakova et al. (2016). Briefly, pair-end reads were merged using fastq-join (Aronesty, 2013). Whole amplicons were processed for bacterial 16S, while the ITS2 region was extracted using ITS Extractor 1.0.8 (Nilsson et al., 2010) before processing. Chimeric sequences were detected using Usearch 7.0.1090 (Edgar, 2010) and deleted, and sequences were clustered using UPARSE implemented within Usearch (Edgar, 2013) at a 97% similarity level. Consensus sequences were constructed for each cluster, and the closest hits at a genus or species level were identified using BLASTn against the RDP (Cole et al., 2014) and Genbank databases (for bacteria) or UNITE (Koljalg et al., 2013) and GenBank for fungi. Sequences identified as non-bacterial or non-fungal were discarded. From 16S rRNA in DNA, bacterial genome count estimates were calculated based on the 16S copy numbers in the closest available sequenced genome as described previously (Větrovský and Baldrian, 2013).

Protein extraction and sample preparation for mass spectrometry analysis

Protein extraction was performed according to the method described by Bastida et al. (2014). Briefly, 4 g of compost were extracted per sample. Sodium dodecyl sulphate (SDS) buffer was added to each sample and incubated at 100ºC for 10 min for cell lysis and proteinases inactivation. Samples were centrifuged at 3000 rpm for 10 min, and the supernatant was transferred to new tubes. The protein precipitation and purification was performed using trichloroacetic acid (TCA) to the supernatant followed by centrifugation at 13000 rpm for 20 min and three acetone washing steps. Protein pellets were suspended in 30 µL 1x Laemmli buffer (Laemmli, 1970), dissolved via ultrasonication and incubated under shaking at 500 rpm at 60 ˚C for 10 min. Then, samples were loaded on SDS gels (4% stacking gel and 12% separating gel) and run at 10 mA. The gels were stained by colloidal Coomassie Brilliant Blue G-250 (Roth, Kassel, Germany). The part of gel containing the protein mixed was cut into one piece, and after being destained, an in-gel tryptic digestion was performed (Jehmlich et al., 2008). The gel bands were washed twice with 200 µL 10mM ammonium bicarbonate in acetonitrile (40%, v/v) for 10 min. Washing solution was removed and samples were dried with 200 µL of acetonitrile for 5 min. Acetonitrile was removed and the gel slices were reduced and alkylated by 30 min incubation with 30 µL 10 mM dithiothreitol in 10mM ammonium bicarbonate and 30 µL 100 mM iodoacetamide in 10 mM ammonium bicarbonate each. Remaining solution was discarded and samples were dried in a vacuum centrifuge followed by incubation with acetonitrile as previously described. Samples were then incubated with 30 µL 5 mM ammonium bicarbonate (containing 0.01 µg μL^{-1} trypsin (Promega)) overnight at 37 ˚C. Finally, the peptides were extracted twice using 30 µL acetonitrile/formic acid (50%/5%, v/v) and concentrated in a vacuum centrifuge. Finally, samples were resuspended in 0.1% formic acid, and desalted and purified by ZipTip® treatment (EMD Millipore, Billerica, MA, USA). Peptides were analyzed by nano-HPLC system Advion NanoMate and Orbitrap Fusion mass spectrometer (Thermo Scientific San Jose, CA, USA). The peptides were eluted over 120 min with a gradient of 2 to 60% solvent (acetonitrile, 0.1% formic acid). MS scans were measured at a resolution of 120,000 in the scan range of 400-1600 m/z.

Database searching and bioinformatic classification of protein groups

LC-MS spectra were searched using the Proteome Discoverer (Thermo Fisher Scientific, v1.4, San Jose, CA, USA). Search settings were: Sequest HT search engine against the UniProt bacteria and fungi database (http://www.uniprot.org/), trypsin (full specific), MS tolerance 10 ppm, MS/MS tolerance 0.02 Da, two missed cleavage sides, dynamic modifications oxidation (Met), static modifications carbamidomethylation (Cys). Only peptides that passed the FDR thresholds set in the Percolator node of <1% FDR q value, and were rank 1 peptide, were considered for protein identification.
In order to assign the protein groups to their phylogenetic origin the searching engine PROPHANE was used (http://www.prophane.de/index.php). The protein abundance was quantified using the average MS1-area of the top-3 peptides. Protein abundances were log2-transformed and median-normalized.

Data analysis

One-way ANOVA followed by the Tukey post-hoc test ($p<0.05$) was used to compare physico-chemical, chemical, microbial activity, suppressive effect and, the relative abundance (phyla and order) data from sequencing and proteomic analysis of composts. Pearson correlations among different parameter and disease suppression were also calculated by SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). In order to compare the phylogenetic and functional structure of microbial communities between composts, principal component analysis (PCA) was carried out with: i) the relative abundance of microbial populations studied by genomics (16S rRNA gene and ITS); ii) the relative abundance of microbial populations studied by metaproteomics; and iii) the abundance of proteins essential for cellular functionalities (COGs).

Results

Composts chemical characteristics, microbial activity and disease incidence

The suppressive effect of the composts against *P. nicotianae* (disease incidence % respect to peat) changed significantly between different composts involved in this study ($F=22.80$; $p<0.05$). Compost C3 showed the highest *P. nicotianae* incidence compared to the other composts (Fig. 1). The EC values were significantly different between composts ($F=131.04$; $p<0.01$) (Table 1): C1 showed the highest values (3.35

ms cm^{-1}) followed by C2 and C3 (2.6 and 1.77 ms cm^{-1} respectively). A significant negative correlation was found (F=-0.826; p<0.01) between disease incidence by *P. nicotianae* and EC. pH values also differed significantly (F=1188,7; p<0.05) between composts, C2 showed highest value (9.4) followed by C3 and C1 (8.75 and 7.50 respectively).

The content of organic carbon (TOC) and total N showed significant changes between composts (Table 1). Composts C2 and C3 showed lower TOC and total N compared to C1 (F=17.58; p<0.05; F=22.59 p<0.05) respectively.

Microbial activity of composts measured by dehydrogenase activity showed significant differences between composts (F=14.82; p<0.05), the highest activity was found in composts C2 and C3 compared to C1 (Table 1).

Composition of total bacterial and fungal community by sequencing

Sequencing analysis of 16S rRNA gene showed 4147 OTUs after clustering at 97% similarity. The relative abundance of microbial populations of the three composts were dominated by *Proteobacteria* (52%), *Bacteroidetes* (21%) and *Actinobacteria* (13%) (Fig. 2).

PCA of 16S rRNA sequences showed that according to factor 1 (55% of the total system) composts separated significantly (p<0.05) in two groups, one group (C1 and C2) and the other group (C3) (Fig. 3). C1 and C2 showed significantly (p<0.05) higher relative abundance of *Proteobacteria* compared to the compost C3 (Fig. 2).

Within the dominant phylum, the most abundant subgroups in *Proteobacteria* were *Gammaproteobacteria* and *Alphaproteobacteria* with almost 85% of *Proteobacteria*. While, *Alphaproteobacteria, Sphingomonadales* and *Rhodobacteriales* increased, *Rhodospirillales* decreased significantly in C1 and C2 (Table S1). Within *Gammaproteobacteria* the relative abundance of *Xanthomonodales* and *Cellvibrionales* were higher in C1 and C2 than in C3, while that of *Nevskiales* was significantly lower (p<0.05) in C1 and C2 (Table S1). Within *Bacteroidetes*, orders *Cytophagales* and *Chitinophagales* showed significant (p<0.05) lower relative abundance and higher relative abundance of *Flavobacteriales* and *Sphingobacteriales* for C1 and C2 (Table S1). Orders belong to *Actinobacteria* as *Micrococcales* increased and for *Acidimicrobiales* decreased significantly (p>0.05) in C1 and C2 (Table S1).

The sequencing of ITS2 region indicated the dominance of Ascomycota (95%) followed by Basidiomycota (3%) and Mucoromycota (2%). Within Ascomycota, *Microascales* and *Sordariales* were the dominant orders. Composts C1 and C2 showed significant lower ($p<0.05$) relative abundance of *Sordariales* than the other compost C3. Also, compost C3, showed significant lower relative abundance of Mucoromycota (*Morteriella)* than the other composts.

Composition of bacterial community through metaproteomics

Metaproteomics allowed the identification of 367 proteins. Proteins from composts were identified (99.6%-100%) as bacterial proteins. As regards of the phylogenetic analysis of proteins, the bacterial composition of compost was dominated by *Proteobacteria* (61%), *Actinobacteria* (20%) and *Thermotogae* (11%) (Fig. 4). C1 and C2 showed higher relative abundance of *Proteobacteria* and *Deinococcus* than C3 (Fig. 4).

Within the dominant phylum (*Proteobacteria*), the most abundant subgroups were Gammaproteobacteria and Alphaproteobacteria with almost (71%). Within *Gamma-proteobacteria* the most abundant bacterial proteins belong to order *Xanthomonadales, Enterobacteriales* and *Pseudomonodales. Xanthomonadales* were higher ($p<0.05$) for C1 and C2, and *Enterobacteriales* and *Pseudomonodales* were lower ($p<0.05$) for C1 and C2 compared to C3 (Table S2). Within *Alphaproteobacteria* the most abundant order was *Rhizobiales*, where C1 and C2 were higher compared to C3. Also, *for Betaproteobacteria* the most abundant order was *Burkholderiales* higher for C1 and C2 compared to C3. The order *Kosmotogales (Thermotogae)* was significantly the highest in C3 ($p<0.05$). Within *Actinobacteria,* order *Micromonosporales* showed higher relative abundance in C1 and C2, and ($p<0.05$) lower in *Chloroflexi (Chloroflexales)* (Table S2).

Microbial functionality

The relative abundance of proteins involved in different functions as cell wall structure (M), carbohydrate and transportation (G), inorganic ion transport and metabolism (P) were significant higher ($p<0.05$) in C1 and C2 than compost C3, while functions as energy production and conversion (C), lipid transport and metabolism (I)

were significantly lower ($p<0.05$) in C1 and C2 than compost C3 (Fig. 5). PCA indicated that the functional structure of C1 and C2 were similar and clearly different to that of C3 (Fig 6).

Correlation of sequencing and metaproteomics data to disease incidence by P. nicotianae

A main objective of this analysis of composts was to correlate taxonomic and functional data with disease incidence by *P. nicotianae* of composts that could be used as bioindicators.

A significant correlation between disease incidence by *P. nicotianae* and PCA 1 (Phylogenetic level) ($F=0.917$; $p<0.05$) and PCA 1 (Functional level) ($F=0.864$; $p<0.01$) was found. At both levels, the relative abundance of *Proteobacteria* showed a significant negative correlation with disease incidence ($F=-0.700$; $p<0.05$) and ($F=-0.762$; $p<0.05$) respectively. PCA1 of COGs showed a significant correlation ($F=0.917$; $p<0.01$) with disease incidence. Between different functions V, C and I showed a positive significant correlation with disease incidence ($F=0.819$; $p<0.01$; $F=0.859$; $p<0.01$; $F=0.935$; $p<0.01$) respectively, and a negative significant correlation with M, G, P, ($F=-0.789$; $p<0.05$; $F=-0.894$; $p<0.01$; $F=-0.902$; $p<0.01$) respectively.

Discussion

It is clear that composts suppressiveness is pathogen-specific and related to the mechanism(s) of disease suppression attributed to either chemical or biological factors of composts (Hadar and Papadopoulou, 2012). Two of the three composts used in the experiment (C1 and C2) were considered suppressive against *P. nicotianae* in pepper, with a disease incidence <50%, and the last, C3, non-suppressive or conductive against *P. nicotianae* in pepper with a disease incidence >50% (Blaya et al., 2015). Between physico-chemical characteristics, the effect of high EC levels has also been exploited in controlling root pathogens belonging to *Phytophthora* spp. on Gerbera (Thinggaard and Anderssen, 1995). We found a negative correlation between EC and disease incidence by *P. nicotianae*, indicating that composts with high levels of EC C1 (3.35 mS cm^{-1}) and C2 (2.26 mS cm^{-1}) can help to control *P. nicotianae*, without interfere seedling growth (Lemaire et al., 1985).

Special attention should be paid on the starting material used for composting process (Castaño et al., 2011). Composts utilized in this study had a different composition of raw materials, and microorganisms (Hoitink and Boehm, 1999; Ishii and Takki, 2003) and some of them could support the compost suppresiveness. Garlic residue is a substance with a broad-spectrum antimicrobial activity against many genera of bacteria and fungi (Adetumbi et al., 1983). Compost C1 included garlic residues (25%), that it would support the above hypothesis related to pathogen suppression. However, compost C3 was not considered suppressive and its composition includes garlic but it was in a lower percentage (3%). This could indicate that not only the type of raw materials, also, the proportion and the mix of raw materials could influence the composts suppressive activity against *P. nicotianae.*

A positive correlations between composts microbial activity and *Phytopthora* spp. or *Pythium* spp. suppression (Ntougias et al., 2008; Blaya et al., 2015) indicated that microbial activity could be used as potential indicator of composts suppressiveness. However, this fact was not consistent with the results obtained in our study. Dehydrogenase activity, a wide recognized indicator of potential microbial activity (Garcia et al., 1993) did not show any correlation with disease incidence. For this reason, exploring the composition and functionality of microorganism from suppressive/non-suppressive composts is important to its management.

The use of high-throughput sequencing technologies has proven as a powerful tool for the identification of the variability of bacterial and fungal composts community (Neher et al., 2013; de Gannes et al., 2013) to manage soil borne pathogens (Mengesha et al., 2017; Blaya et al., 2016). Moreover, according to Bastida et al. (2016), functional and phylogenetic analyses of proteins in soils, complemented DNA-based community analyses and allowed a distinction of lifestyles that could not be achieved solely by the amplification of taxonomical genes. In this study, the suppression of various composts against *P. nicotianae* in pepper plants by sequencing and metaproteomics were characterized to elucidate difference between suppressive/non-suppressive composts.

In our study, we found differences in the abundance of microbial populations of the different compost, both through amplicon sequencing and metaproteomics.

16S rDNA amplicon sequencing indicated that *Proteobacteria* (*alpha- and gamma-*), *Bacteroidete and Actinobacteria* are the dominant phyla consistent with previous

composts sequencing studies (Danon et al., 2008; Partanen et al., 2010, de Gannes et al., 2013). While by metaproteomic analysis the dominant phyla were *Proteobacteria* (*alpha- and gamma-*), *Actinobacteria*, and *Thermothogae* consistent also with compost protein-based analysis (Liu et al., 2015). 16S rDNA gene sequencing and the functional analysis of proteins allowed deciphering the microbial key players that can be potentially involved in suppressive composts.

The *Proteobacteria* encompass an enormous level of morphological, physiological and metabolic diversity, and are of great importance to global carbon, nitrogen and sulfur cycling (Kersters et al., 2006). *Proteobacteria* species were significant negative correlated with disease incidence, indicating their dominance and a link between taxonomy and functionality in suppressive composts (Mehta et al., 2016; Blaya et al., 2016), considering that it could be an indicator of suppressive composts. Several orders of *Proteobacteria* were identified by sequencing and protein analysis and could have been involved in suppressive activities towards soil pathogens. The abundance of *Xanthomonadales* or *Burkholderiales* was higher, in suppressive composts, which, harbor genera and species with activity against plant pathogenic fungi (Postma et al., 2010). *Pseudomonadales* has been also identified as antagonists of soil borne pathogen (Gonzalez -Sanchez et al., 2010) and induce systemic resistance to plants (Tian et al., 2007). Proteins matched to *Rhizobiales* were higher in suppressive composts, considering that several rhizobial strains are reported to have plant growth induction, biocontrol properties and the resistance spectrum available against various abiotic stresses on a variety of agricultural crops (Gopalakrishnan et al., 2015).

Actinobacteria harbor populations with activity against plant pathogen fungi (Postma et al., 2010). Proteins of *Micromonosporales*, an *Actinobacterial* order, are principally present in suppressive composts compare to non-suppressive. Indeed, it has also been shown that non-*streptomycetes Actinobacteri*a are a useful source of novel bioactive compounds (Stackebrandt and Ebers, 2006; Kurtboke, 2012.). The AntiBiotic Literature (ABL) database demonstrates that members of the family *Micromonosporaceae* account for the highest proportion (38%) of bioactive producing strains among non-streptomycete families.

Proteins associated to carbohydrate process, cell wall structure and inorganic ion transport and metabolism were deeply involved in suppressive composts compare to

non-suppressive. Data suggested that these COGs could be indicators of suppressive composts.

Our study showed a significant higher amount of bacterial proteins relative to nearly non-existence of fungi proteins. This is probably related to the poor coverage of fungi genomes in the general metagenome databases used for protein searches (Liu et al., 2015).

Conclusions

Our study concludes that there are differences between suppressive and non-suppressive composts at phylogenetic level (sequencing) and at functional level (analysis of COGs). It is also important to highlight the differences between DNAs and protein analyzes. *Proteobacteria* could be a good indicator of composts suppressivity due to their high correlation with disease incidence by *P. nicotianae*. The presence of certain microorganisms can contribute to the compost suppressivity although they are not determinants of the same. Other types of physico-chemicals factors such as EC may driver the suppressiveness of composts. The development of metagenome databases, with a higher number of fungal genomes, would extent the knowledge on the biological mechanisms of supressiveness mediated by fungi.

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1 **Table 1**. Chemical characteristic of composts and peat used as organic substrates

	C1	C2	C3	Peat
pH	7.50±0.03	9.42±0.04	8.75±0.06	5.5±0.05
EC (mS cm^{-1})	3.35±0.02	2.6±0.16	1.77±0.13	2.0±0.06
TOC (g kg^{-1})	432.8±5.0	390.1±5.5	381.6±6.1	450±4.5
Nt (g kg^{-1})	33.01±0.05	26.8±0.06	28.5±0.08	13.0±0.05
Pt (g kg^{-1})	4.40±0.04	5.7±0.05	4.7±0.06	0.3±0.02
Kt (g kg^{-1})	16.6±0.01	38.7±0.02	18.0±0.04	0.6±0.01
D. A (mg INTF g^{-1} soil h^{-1})	53.16±9.03	81.65±7.12	87.23±8.38	nd

2 D.A: Dehydrogenase Activity
3 Nd: not detected

4

Fig. 1. Disease incidence (%) of different composts (disease incidence % of peat was 90%)

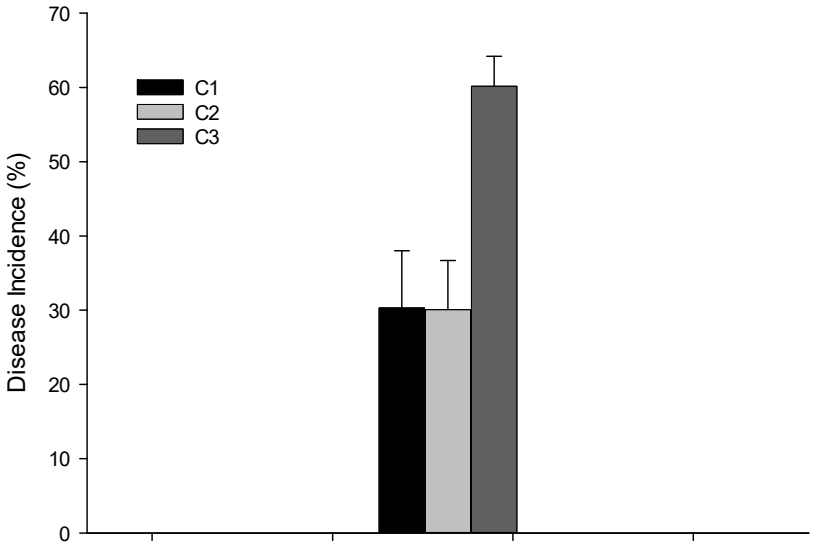


Fig. 2. Relative abundance of different bacterial phyla (16S rRNA) within different composts (relative abundance >0.5%)

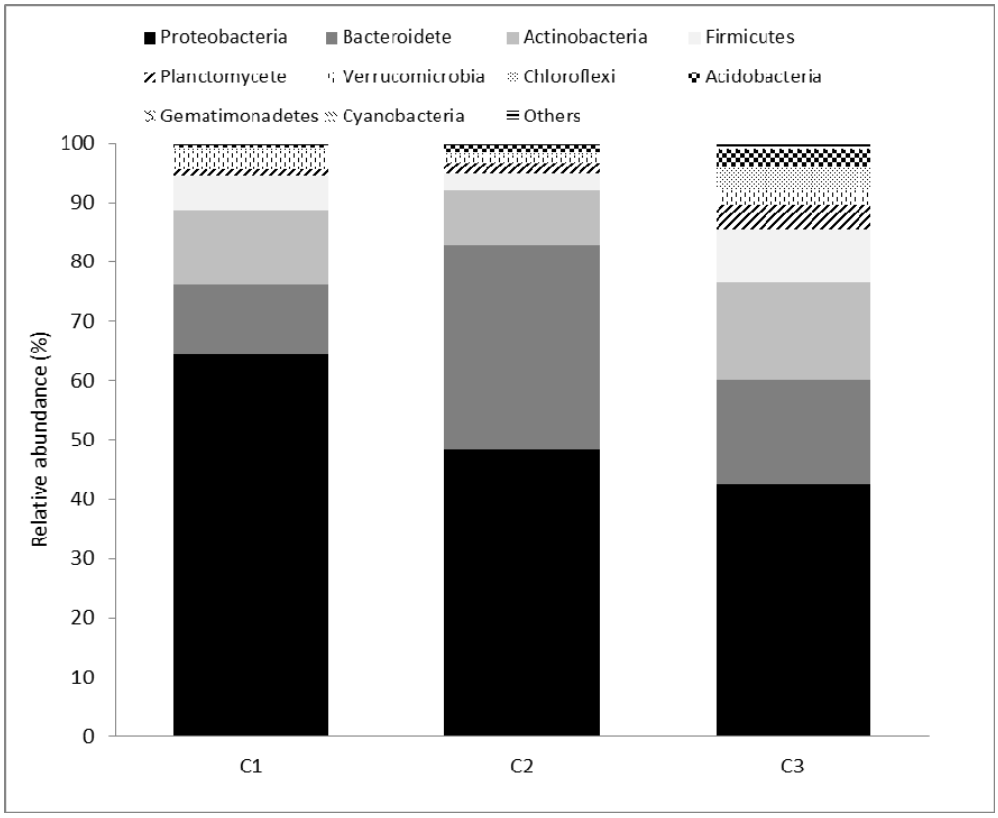


Fig. 3. Principal component analysis of bacteria phyla based on relative abundance of the phyla

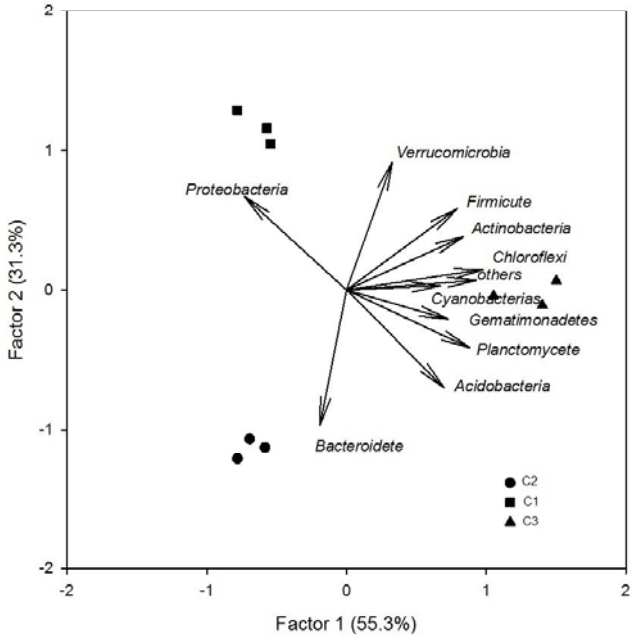


Fig. 4. Phylogenetic assignment of bacterial proteins in composts (relative abundance >0.5%)

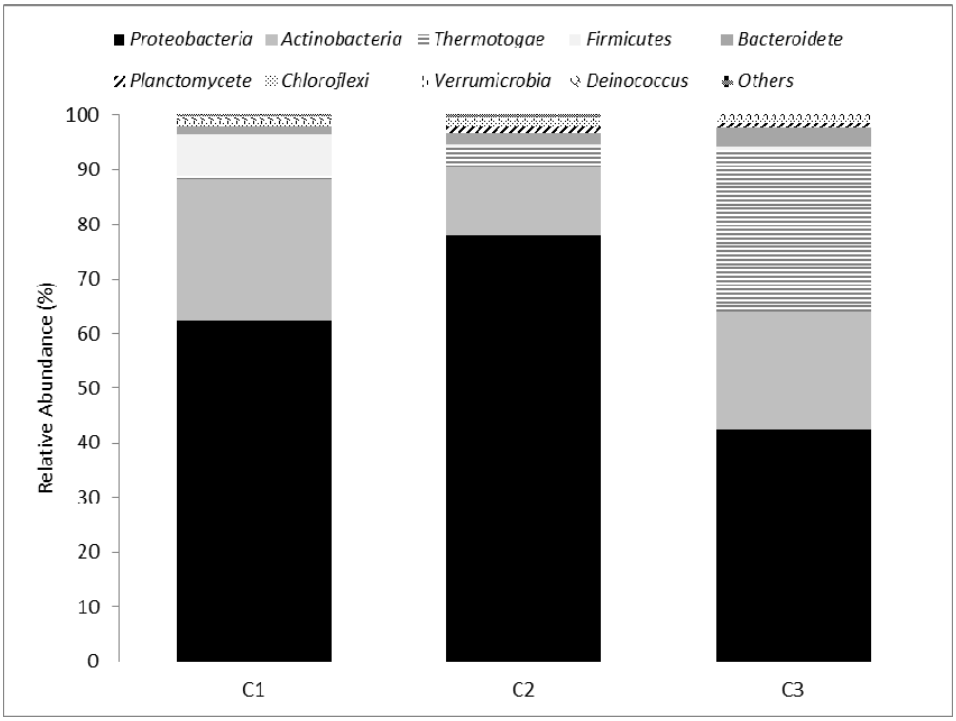


Fig. 5. Functional classification of microbial proteins in composts. (% relative COGs >2%). Abbreviations: M (Secondary metabolites biosynthesis, transport and catabolism); O (Transcription); V (Defense mechanism); J (Nucleotide transport and metabolism); K (Posttranslational modification, protein turnover, chaperons); C (Cell cycle control, cell division, chromosome partitioning); ET (Amino acid transport and metabolism-Signal transduction mechanisms); G (Energy production and conversion); P (Translation, ribosomal structure and biogenesis); I (Inorganic ion transport and metabolism)

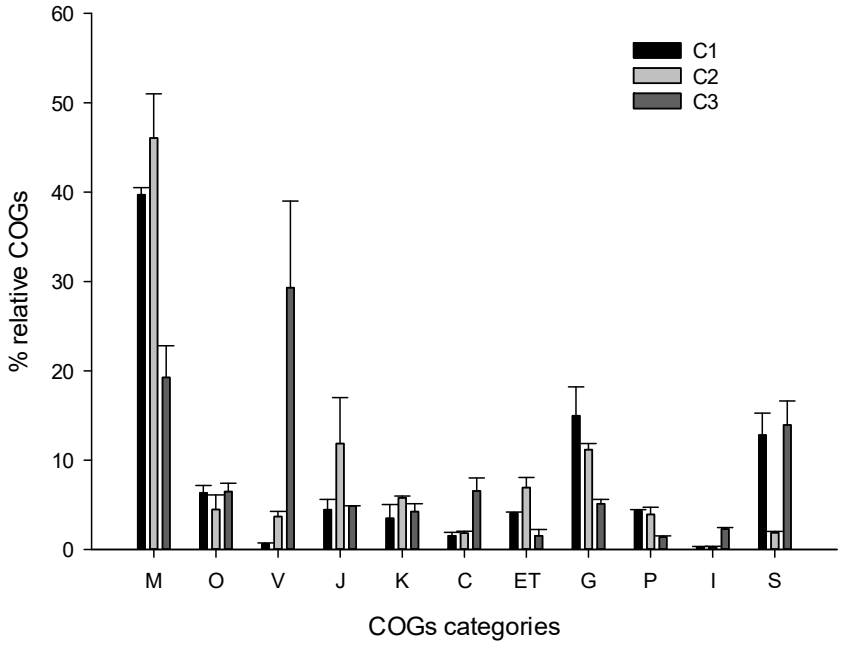
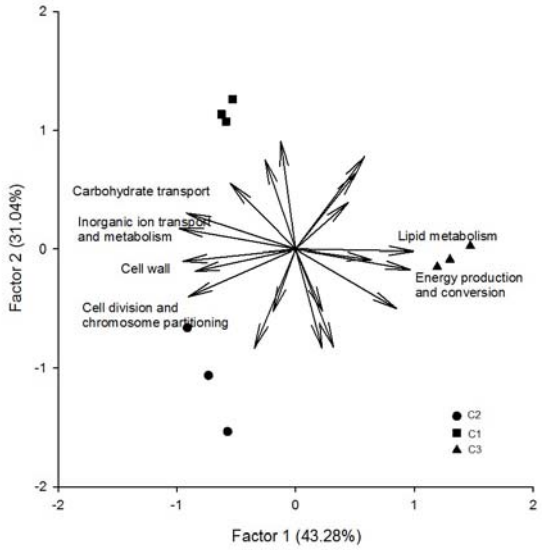


Fig. 6. Principal Component analyses of microbial protein-functional groups (COGs) of composts



In vitro elucidation of suppression effects of composts to soil borne pathogen *Phytophthora nicotianae* on pepper plants using 16S amplicon sequencing and metaproteomics.

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Supplementary Table Legends

Supplementary Table S1. Most abundant orders for bacterial community (>2%) by 16S rRNA analysis.

Suppelemtary Table S2. Most abundant order for active bacterial community (>2%) by protein analysis.

Supplementary Table S1. Most abundant orders for bacterial community (>2%) by 16S rRNA analysis.

Phylum	Order	C1	C2	C3
AlphaProteobacteria	*Rhizobiales*	14.67±0.96	4.35±0.62	6.88±0.34
AlphaProteobacteria	*Rhodobacterales*	1.39±0.17	3.06±0.39	1.20±0.18
AlphaProteobacteria	*Rhodospirillales*	1.21±0.17	0.96±0.05	3.93±0.30
AlphaProteobacteria	*Sphingomonadales*	12.36±1.16	8.15±0.49	2.23±0.48
BetaProteobacteria	*Burkholderiales*	3.53±0.20	0.21±0.08	1.49±0.16
Gammaproteobacteria	*Nevskiales*	2.86±0.30	2.30±0.58	4.30±0.24
Gammaproteobacteria	*Pseudomonadales*	4.19±0.07	2.18±0.18	2.53±0.10
Gammaproteobacteria	*Xanthomonadales*	16.27±0.86	13.86±3.06	5.09±0.59
Gammaproteobacteria	*Cellvibrionales*	1.96±0.09	4.99±0.76	0.83±0.21
DeltaProteobacteria	*Myxococcales*	2.12±0.21	4.58±0.46	3.37±0.55
Bacteroidetes	*Chitinophagales*	1.99±0.18	2.42±0.31	*3.38*±0.58
Bacteroidetes	*Sphingobacteriales*	5.02±0.88	4.68±1.17	0.55±0.09
Bacteroidetes	*Cytophagales*	3.36±0.26	3.73±0.73	*12.09*±2.03
Bacteroidetes	*Flavobacteriales*	1.27±0.21	22.87±2.21	0.76±0.10
Actinobacteria	*Streptosporangiales*	2.50±0.66	0.18±0.13	2.93±0.59
Actinobacteria	*Micrococcales*	4.06±0.16	4.71±0.49	1.71±0.26
Actinobacteria	*Acidimicrobiales*	0.67±0.10	2.38±0.30	*5.52*±0.16
Firmicutes	*Bacillales*	5.13±0.66	2.27±0.43	6.27±0.55

Supplementary Table S2. Most abundant order for active bacterial community (>2%) by protein analysis.

Phylum	Order	C1	C2	C3
GammaProteobacteria	*Xanthomonadales*	6.51±2.23	17.24±5.16	2.0±0.57
GammaProteobacteria	*Enterobacteriales*	4.21±0.92	5.12±0.91	7.82±1.23
GammaProteobacteria	*Pseudomonadales*	1.75±0.62	0.46±0.13	3.39±0.80
AlphaProteobacteria	*Rhizobiales*	17.28±2.50	31.82±4.23	14.06±5.01
AlphaProteobacteria	*Rhodobacterales*	2.02±0.81	1.65±0.11	1.29±0.38
BetaProteobacteria	*Burkholderiales*	13.01±4.84	7.88±0.98	6.72±1.74
BetaProteobacteria	*Rhodocyclales*	3.13±0.77	1.38±0.56	1.11±0.45
DeltaProteobacteria	*Myxococcales*	2.41±1.03	2.94±0.47	1.78±0.52
Actinobacteria	*Streptosporangiales*	12.64±1.96	4.84±1.42	9.58±0.29
Actinobacteria	*Corynebacteriales*	1.34±0.48	0.91±0.35	*3.74±2.23*
Actinobacteria	*Micromonosporales*	9.85±1.30	5.23±0.82	3.56±0.84
Actinobacteria	*Streptomycetales*	2.92±0.75	0.00±0.00	*2.55±0.35*
Thermotogae	*Kosmotogales*	0.45±0.29	2.79±1.65	28.62±10.08
Chloroflexi	*Chloroflexales*	0.50±0.16	0.94±0.47	2.04±0.33
Firmicutes	*Clostridiales*	6.30±1.61	0.38±0.41	0.37±0.10
Deinococcus-Thermus	*Deinococcales*	2.47±0.57	0.00±0.00	0.00±0.00