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This is a pre print version of the following article:

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1891848 since 2023-02-13T07:30:57Z

Published version:

DOI:10.17660/ActaHortic.2020.1270.13

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12 October 2024

Microbial communities involved in suppression of Fusarium wilt of lettuce by a municipal biowaste compost

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Abstract

Compost suppressiveness depends primarily on microbiological composition and antagonists can be isolated from high quality composts. The objective of the present work was to evaluate the suppressive effect of compost against *Fusarium oxysporum* f. sp. *lactucae*, and to study the microbial communities involved. A compost from municipal biowastes that showed a good suppressive activity in previous trials was added at 1% to a steamed sandy soil inoculated with the pathogen. Compared to the same soil not treated, compost showed a 40% disease control of *Fusarium* wilt of lettuce. Microbial activities, bacterial and fungal concentrations were quantified and correlated in a Principal Component Analysis in order to clarify the correlation between the variables and compost suppressiveness. Samples taken from the rhizosphere of plants grown in suppressive media had highest total enzymatic activity and highest concentration of total fungi. The comparison of DGGE profiles of microbial populations revealed a greater diversity in the fungal community than that of bacteria. *Pseudomonas* sp., among bacteria, and *Simplicillum lamellicola*, among deuteromycetes, were detected only in the rhizophere of plants treated with 1% compost, indicating that they may play an active role in disease suppressiveness.

Key words: Pseudomonas, DGGE, rhizosphere, *Fusarium oxysporum* f. sp. *lactucae*

INTRODUCTION

Lettuce is a high-value crop which cultivation gained recently a significant economic importance, especially in Italy, due to the increasing production and marketing of ready-to-eat salads.

First observed in Japan in 1955, *Fusarium oxysporum* f. sp. *lactucae* has spread also in USA, South America and Europe (Italy, Portugal, France, The Netherlands…) becoming the most important soil-borne pathogen affecting lettuce worldwide (Matheron and Gullino, 2012). The pathogen is specific to the host lettuce, it causes leaf's yellowing and wilting and can be seedborne (Garibaldi *et al.*, 2004a).

The management of Fusarium wilt of lettuce should consider the use of healthy seeds and/or seed treatments to reduce the risk of introduction of the pathogen in the soil. Other strategies reduce the inoculum and pathogen survival, such as fumigation, steaming, flooding, biocontrol and solarization. However, due to regulatory, technical and economic constraints, these practices are not feasible and applicable to all cases

and situations. Lettuce cultivars differ in susceptibility, only few of them showed some levels of resistance, and the genetic basis for resistance to *F. oxysporum* f. sp. *lactucae* is not yet fully understood (Garibaldi et al, 2004b; Gordon and Koike, 2015; Pintore et al., 2017).

Compost represents an opportunity to control soil-borne pathogens and its suppressiveness depends primarily on microbiological composition and varies according to the pathosystem and the type of compost that is applied (Termorshuizen *et al.,* 2006; Pugliese *et al.,* 2015). Antagonists can also be isolated from high quality composts (Pugliese *et al.,* 2008), but the entire microbial community is generally involved in suppressiveness and compost amendment can influence the size or the composition of the soil microbial communities leading to the control of plant diseases (Zaccardelli *et al.,* 2013).

Due to the complexity of interactions between soil microorganisms and plants, different and combined approaches must be adopted to study the mechanisms that drive compost suppressiveness, looking also at the rhizosphere. Beside enzymatic assays, which are still considered useful to quantify microbial activities, more informative techniques like metagenomic tools are the most promising (Zhou *et al.*, 2015).

The objective of the present work was to evaluate the suppressive effect of compost against *Fusarium oxysporum* f. sp. *lactucae*, also analyzing the microbial communities involved in the suppression.

MATERIALS AND METHODS

Preparation of trials and samples

A commercially available compost prepared from green wastes and anaerobic digested municipal biowastes was used because it had shown a good plant disease suppressiveness in previous trials (Pugliese *et al.,* 2007). The compost was added at 0 and 1% v/v to a sandy soil, previously steamed, and use to carry out pot experiments. Part of the soils (mixed and not mixed with the compost) was inoculated with a talc of clamidospores of *Fusarium oxysporum* f. sp. *lactucae* to reach a final concentration of $5x10^4$ UFC/g.

The roots of one-month-old lettuce seedlings, cv. Crispilla Bianca, were washed to remove any substrate debris and 10 seedlings per treatment were transplanted in 1l pots containing the soils. Five pots were used for each treatment.

Plants were maintained in a climatic chamber under controlled humidity and at an optimum temperature for the pathogen (80–90% HR, 27°C), with a randomized experimental block design.

Evaluation of compost suppressiveness and microbial community analysis

Plant disease assessments were carried out weekly using a disease scale of 5 points, ranging from 0 (healthy plant) to 4 (dead plant). The suppressive capacity (SC%) and the area under disease progress curve (AUDPC) were calculated according to Termorshuizen et al. (2006).

Soil and rhizosphere samples were collected at the time of plant transplanting and 30-35 days after transplanting. Microbial activity was estimated in the samples by the means of fungal and bacterial Colony Forming Unit (CFU) counts, enzymatic assays (FDA, according to Ryckeboer et al. 2003, and β-glucosidase, according to Andres Abellan et al. 2011) and DGGE technique (fungal DNA was amplified according to the protocol from Gao et al. 2012, while for bacteria DNA was applied the protocol of Nocker et al. 2007).

Statistical analysis

Statistical analysis was carried out using SPSS 21.0. After assessing the normality and homogeneity of variances, the data of the two experiments were pooled together. ANOVA and Tukey's "HSD" post-hoc tests were used, with a significance defined at the $P < 0.05$ level. Principal Component Analysis (PCA), which was elaborated with PAST (Paleontological Statistics software package for education and data analysis; Hammer et al. 2001).

RESULTS AND DISCUSSION

Disease severity was 2.85±0.37 in the untreated soil and 1.55±0.45 with the addition of 1% compost to the soil. A disease reduction of 41% was observed, with significant differences in the AUDPC values (Fig. 1).

At the beginning of trial, FDA in the healthy control was significantly the lowest and β-glucosidase was significantly higher in the 2 compost treated soils. No significant differences were observed in the total bacteria and fungi.

At rhizosphere level, at the end of the trial, FDA in the inoculated and compost treated soil was significantly the highest, and β-glucosidase, total bacteria and total fungi in the same soil were higher than inoculated untreated soil. This might be the consequence of an enhanced carbon and nutrient allocation in the rhizosphere due to better growth conditions and lower disease.

According to the DGGE profile of the bacterial population, *Pseudomonas* sp. was found only in the rhizosphere of the plants grown in soil amended with 1% compost, suggesting an involvement in disease suppression (Fig. 2). *Fusarium* and other microrganisms, such as *Metschinkowia chrysoperlae*, *Dyctionema sericeum* and *Peziza basisiofusca* were amplified (Fig. 3). Among the fungi, *Simplicillium lamellicola* was the only species detected in the rhizosphere of plants treated with 1% compost, thus suggesting a possible role in the process of disease suppression.

CONCLUSIONS

The use of compost can be a suitable strategy for controlling important soil-borne diseases on vegetable crops, like Fusarium wilt of lettuce. Compost showed to promote a higher bacteria and fungi population and enzymatic activity at both soil and rhizosphere level, as well as the presence of specific groups of microorganisms, like *Pseudomonas* sp., well-know for their capacity to control plant pathogens.

Future researches may focus on analysing microbial communities involved in compost suppression considering also other suppressive and not suppressive composts, and different soils, in order to identify the common microbiological characteristics responsible for suppressiveness.

ACKNOWLEDGEMENTS

This research was carried out with the support of the University of Torino (ex-60% project). The authors would like to thank S. Franceschini for her technical assistance.

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Figures

Fig. 1. Mean AUDPC values for plants inoculated and grown in non-amended soil (0%) and soil amended with 1% of ACM compost. Different letters above the column indicate significant ($p < 0.02$) differences between groups of values (Mann–Whitney test).

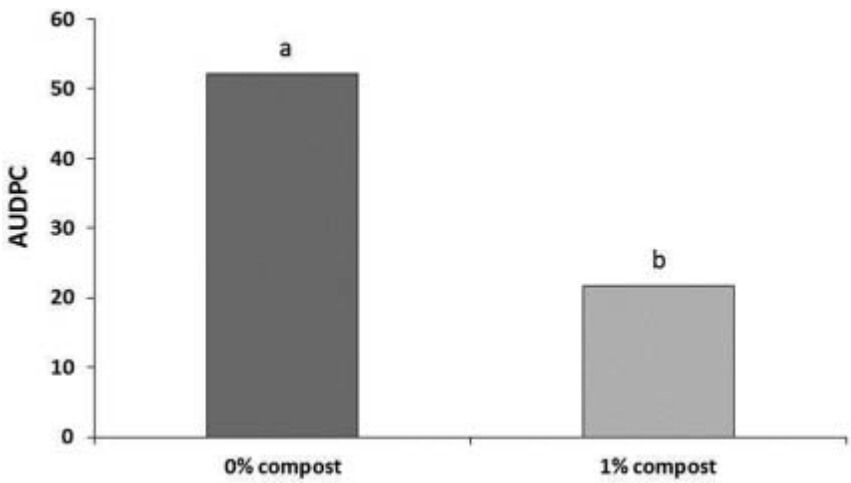
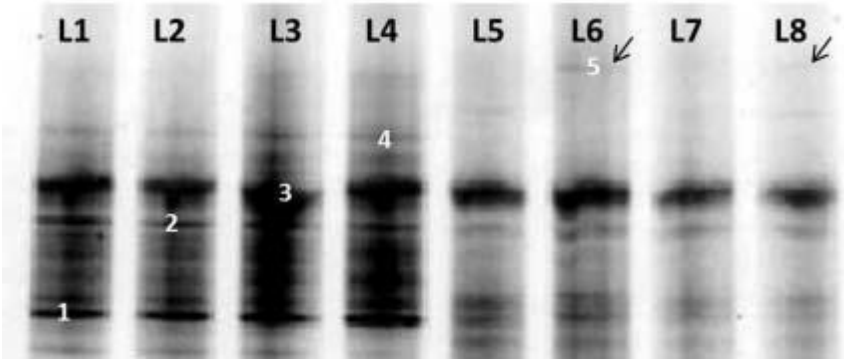
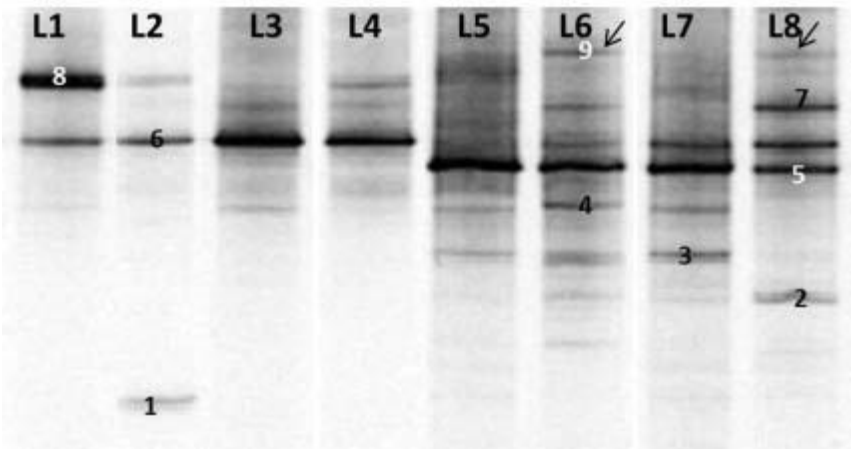


Fig. 2. DGGE profiles of bacterial community at the Time zero (Ti) lines 1 to 4 and at the end of the experiment (Tf) lines 5 to 8.



Treatments:
(L1) Ti–not inoculated–0% compost–no plant;
(L2) Ti–not inoculated–1% compost–no plant;
(L3) Ti–inoculated–0% compost–no plant;
(L4) Ti–inoculated–1% compost–no plant;
(L5) Tf–not inoculated–0% compost–rhizophere;
(L6) Tf–not inoculated–1% compost–rhizophere;
(L7) Tf–inoculated–0% compost–rhizophere;
(L8) Tf–inoculated–1%compost–rhizophere.
Sequences: (1) *Flavobacterium tiangeerense*; (2) Uncultured bacterium; (3) *Flavobacterium xinjangense*; (4) *Flavobacterium* sp.; (5) *Pseudomonas* sp.

Fig. 3. DGGE profiles of fungal community at the Time zero (Ti) lines 1 to 4 and at the end of the experiment (Tf) lines 5 to 8.



Treatments:
(L1) Ti–not inoculated–0% compost–no plant;
(L2) Ti–not inoculated–1% compost–no plant;
(L3) Ti–inoculated–0% compost–no plant;
(L4) Ti–inoculated–1% compost–no plant;
(L5) Tf–not inoculated–0% compost–rhizophere;
(L6) Tf–not inoculated–1% compost–rhizophere;
(L7) Tf–inoculated–0% compost–rhizophere;
(L8) Tf–inoculated–1%compost–rhizophere.
Sequences: (1) *Metschinkowia chrysoperlae*; (2) Uncultured fungus; (3) Ascomycete; (4) *Verticillium* sp.; (5) *Verticillium* sp.; (6) *Fusarium oxysporum*; (7) *Peziza basidiofusca*; (8) *Dyctionema sericeum*; (9) *Simplicillium lamellicola.*