Effects of three fungicides used in organic fruit production on the bacterial physiological activity in compost tea

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Abstract

Vermicompost tea contains microbes with potential antagonistic activity against plant pathogenic fungi. Compost tea could therefore be of use in organic fruit production in addition to common fungicides. We report on the effects of four different fungicides used in organic fruit production on the bacterial community in vermicompost tea extract, evaluated with microbial metabolic fingerprinting. Cuprofor® remarkably reduced the diversity of the bacterial community in vermicompost tea, while Sulfolac® and VitiSan® seem to have no suppressive effect.

Keywords: compost tea, BIOLOG, organic fruit production, fungicides

Introduction

In organic fruit growing only a small number of plant protection agents are allowed. Most of them are less efficient than the products used in conventional farming. Therefore there is a need for substances with a positive effect on plant vitality.

Compost teas have been shown to suppress different foliar diseases when applied directly to foliage (reviewed by Scheuerell & Mahaffee, 2002; 2004). Vermicompost is a compost product obtained from organic waste degraded by earthworms, and its watery extract is used as vermicompost tea. Vermicompost has been shown to contain high numbers of chitinolytic bacteria that show antagonistic activity against plant pathogenic fungi and could potentially be used as biocontrol agent in plant protection (Yasir *et al.*, 2009).

It is a common practice in organic fruit production to apply fungicides and plant strengtheners at the same time; the agents are often combined in the tank of the spraying machine. Nevertheless the impact of commonly used fungicides on the beneficial microflora of plant strengtheners like compost tea when combined prior to application is not fully known.

In this work, we tested if the four currently most important fungicides (calcium polysulfid, soluble sulphur and copper oxychloride, potassium hydrogencarbonate) used in organic apple production could be combined with vermicompost tea without severely harming the beneficial microflora present in the compost tea.

Material and Methods

46.4 g of compost tea ("Grüner Daumen Komposttee", company Ja!Natürlich, Austria, CT) were immersed with two liters of tap water (ca. 10 °C) and incubated at 20 °C for 24 hours. Afterwards the compost tea was filtered through a paper filter (Schleicher & Schüll 595 1/2, Ø 240 mm). Aliquots were added to four different fungicide substances, suitable for application in organic fruit production, so that the final concentration of the substance in the compost tea extract corresponded to the commonly applied concentration used by apple growers or the recommendations of the manufacturer (Table 1).

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The mixtures were incubated one hour at room temperature and aliquots of 150 µl were then added to wells of the BIOLOG GN2 MicroPlates and EcoPlates (Biolog, USA).

Table 1: Origin, name and concentration of the tested fungicides.

Product Name	**Company**	**Active substance (abbreviation)**	**Conc. [%]**
Cuprofor® flüssig	Kwizda Agro, Austria	Copper Oxychloride (CO)	0.02
Polisolfuro di calcio	Polisenio, Italy	Calcium Polysulfid (CP)	1.00
Sulfolac WG (DF)	Agrostulln, Austria	Soluble Sulphur (SS)	0.30
VitiSan	Biofa, Germany	Potassium Hydrogencarbonate (PH)	0.50

The BIOLOG systems EcoPlateTM and GN2 MicroPlateTM are provided for fast microbial community profiling via carbon source utilization ("metabolic fingerprint"). The samples are directly applied to the microtitre plates and the respiratory activity of the microorganisms utilizing the carbon source causes a color change through the reduction of a tetrazolium dye included in the test-system.

For each substance (and control) two EcoPlates (31 carbon sources in three repetitions per microtiter plate) and two GN2 MicroPlates (95 different reactions per microtiter plate) were used. The plates were incubated following the exact protocols of the test-kits (BIOLOG-Gram, 2001; BIOLOG-Microbial, 2007).

Plate reads were done on a Biolog MicroStation-reader after 4 h, 24 h, 48 h and 120 h of incubation at 20 °C (EcoPlates) or 30 °C (GN2-Plates). The OD-results were grouped into 3 classes: 0: no reaction, 1: intermediary and 2: positive reaction. The classified data were analyzed based on a cluster analysis according to Sneath & Sokal (1973) applying the software BIONUMERICS (Version 6.6.4; Applied Maths, Belgien, 2011). The degree of similarity of the measured community-level physiological profiles was calculated using the defined algorithm "simple matching". The results of the cluster analysis are displayed in a dendrogram showing the similarity degree of each sample analyzed.

Results

Evaluating all four incubation times, the biggest change in color development could be detected between 4 h and 24 h of incubation. In the dendrogram of the EcoPlate results after 24 h of incubation (Figure 1), the variants PH + CT, SS + CT and the control (CT only) cluster at a similarity of about 90 %, while CO + CT clusters together with these three variants only at about 75 % similarity. CP was very different with a cluster similarity of only 50 % with all other variants. The dendrogram of the GN2 MicroPlates confirms these results (Figure 2). The microbial metabolic fingerprint of CO + CT thus indicates a strong reduction of bacterial activity compared to the control, while PH and SS seem to have no suppressive effect on the physiological activity to the original bacterial community in the CT. Calcium polysulfide (CP) obviously interacted with the BIOLOG system, since the blank (only water, no carbon source) also showed the color-change of the tetrazolium dye. CP seems to reduce the tetrazolium dye without microbial respiratory activity, but does not react in the same way with all wells and carbon sources resp. on the test plates.

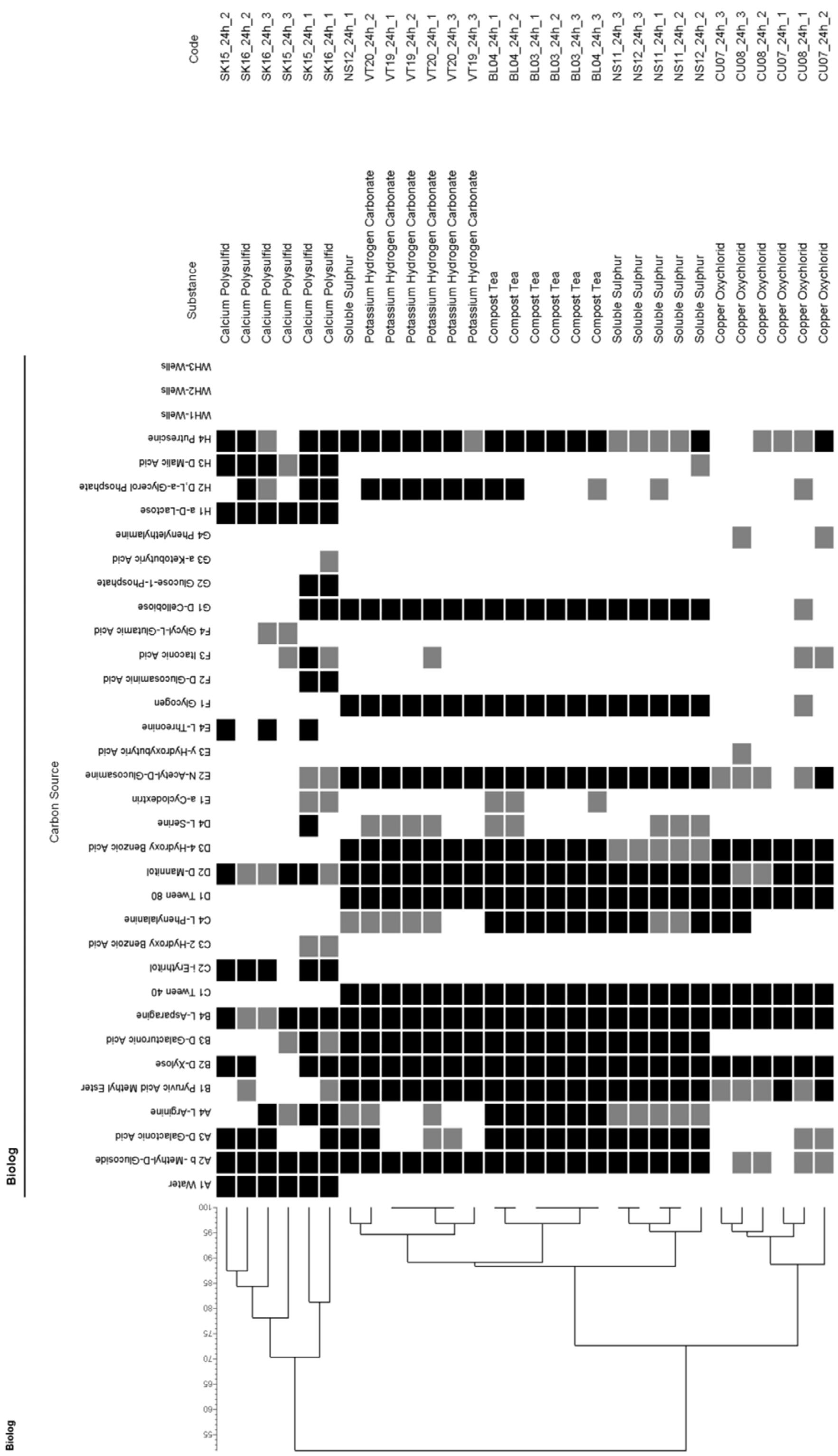

Figure 1: Biolog ECO-Plates after 24h incubation: Dendrogram of cluster analysis (left) combined with an overview of plate readings (middle). The dendrogram shows the degree of similarity between the samples in % and groups similar samples together. The middle horizontal axis indicates the carbon sources on the plate, the vertical axis on the right shows the substance names and the incubation time. Color code of OD-results: white = 0 = negative reaction, grey = 1 = intermediate, black = 2 = positive reaction.

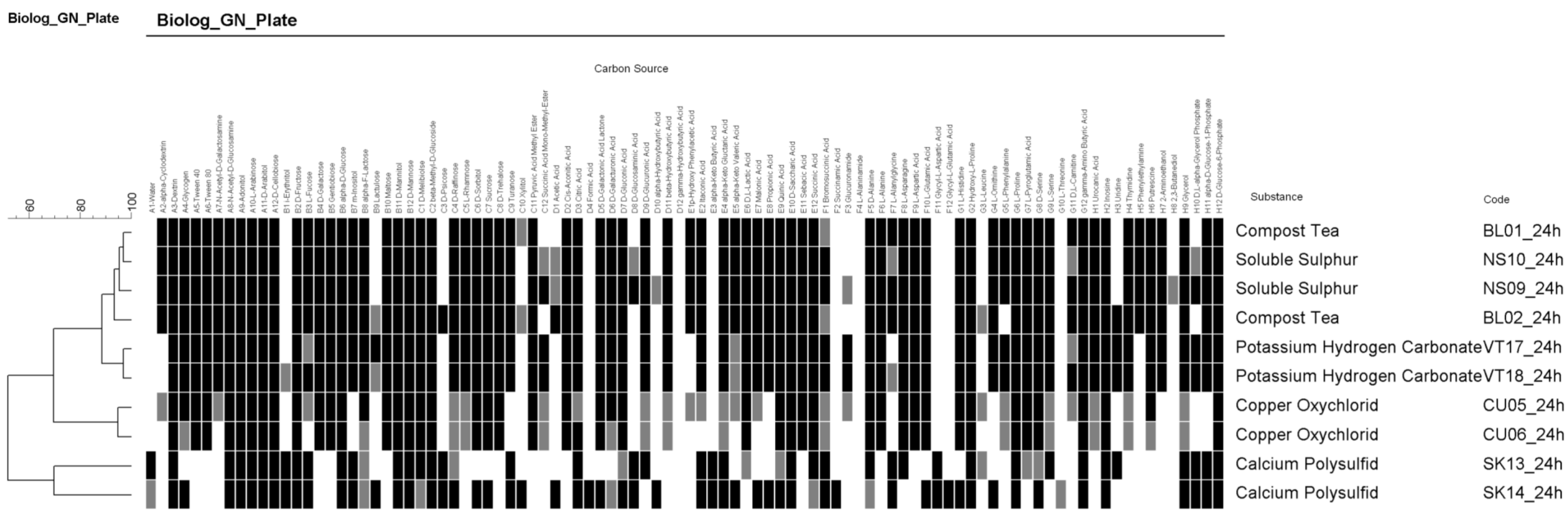

Figure 2: Biolog GN2-Micro Plates after 24h incubation: Dendrogram of cluster analysis (left) combined with an overview of plate readings (middle). The dendrogram shows the degree of similarity between the samples in % and groups similar samples together. The middle horizontal axis indicates the carbon sources on the plate, the vertical axis on the right shows the substance names. Color code of OD-results: white = 0 = negative reaction, grey = 1 = intermediate, black = 2 = positive reaction.

Discussion

The multiple reactions of compost tea extract (control) with the carbon sources in the BIOLOG system confirm that the compost tea contains a very diverse bacterial community. Yasir et al. (2009) report on the bacterial composition of vermicompost via sequencing a 16S rRNA clone-library. The library included clones of Bacteroidetes (31.2 %) γ-Proteobacteria (21.3 %), α-Proteobacteria (17.0 %), β-Proteobacteria (9.6 %), Actinobacteria (6.4 %), Planctomyces (4,3 %) and four unclassified bacterial clones.

CO was shown to influence microbial metabolism in compost tea, while SS and PH don't have any influence. SS and PH could be applied together with CT, without reducing the artisanal microflora of the CT. The advantage of this approach is the combination of the necessary fungicide with the additional fertilizing and antagonistic microbial effects in the CT in only one application step, thus reducing production costs.

CP presumably has been interacting with the BIOLOG system. Therefore the significant reduction in respiratory activity in these plates compared to the control is probably an artefact and not necessarily due to the actual inhibition of bacteria in the compost tea by the substance. In another experiment, CP was shown to slightly inhibit the growth of specific yeasts used as effective microorganisms (EM), while no inhibition was seen with CO or SS (Spornberger *et al*., 2008).

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