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Effect of Chinese medicinal herbal residues on microbial community succession and anti-pathogenic properties during co-composting with food waste

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HIGHLIGHTS

- Chinese medicine herbal residue can be a potential bulking agent/co-substrate.
- CMHR did not inhibit the microbes but influenced the diversity and dominance.
- Food waste–CMHR co-compost showed inhibitory activity against two fungal pathogens.
- CMHR-born active ingredients are the main cause of inhibition.
- Increased population of microbial antagonists contribute to the inhibitory activities.

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ABSTRACT

This study investigated the antimicrobial properties of Chinese medicinal herbal residues (CMHRs) during its co-composting with food waste (FW) in two different ratios along with a control. Inhibition on total microbial population were assessed while the numerically dominant microbes were isolated and their antagonistic effects were assessed. Results indicate that the active ingredients persist in the composting mass did not affect the microbes unspecifically as revealed from almost similar bacterial and fungal populations. Rather specific inhibitory activities against *Alternaria solani* and *Fusarium oxysporum* were observed. Apart from the CMHR-born active compounds, CMHR-induced changes in the antagonistic and mycoparasitic abilities of the bacteria and fungi also contribute to the specific inhibition against the tested pathogens. Therefore use of CMHRs during the composting of CMHRs enhances its antipathogenic property resulting in an anti-pathogenic compost.

1. Introduction

Soil-borne pathogens are the main threat to agricultural crop productivity and yield. Among all phytopathogens, *Alternaria solani* and *Fusarium oxysporum* are two common fungi that cause early blight and vascular wilt in potato/tomato plants, followed by multiple phytopathogenic infections leading to plant damping-off (Roncero et al., 2003). Application of commercial fungicides to control these phytopathogens has serious negative effects on both environmental and human health. From last decade, research on developing bioagents in the form of composts (e.g., citrus compost) to control *F. oxysporum* causing vascular wilt or *Oidium murrayae* causing powdery mildew, thereby improving the crop yield of plants such as melon, cucumber, pumpkin and eggplant (Bernal-Vicente et al., 2008; Chu et al., 2006). The phytopathogens *F. oxysporum* and *A. solani* incur heavy damage globally due to the broad range of host species (Champeil et al., 2004). They cause early blight and stem lesions in common-cultivable plants such as tomato and cabbage. Thus, if compost with potential antipathogenic properties was developed to control *F. oxysporum* and *A. solani*, then the productivity of the plants can be increased that also reduce the dependence on the synthetic fungicides. Abiotic characteristics of soil, such as pH and nutrient contents can influence the activity of bioagents; however, the antagonism and mutualism among the microbes are the two critical interactions affecting the performance of the bioagents which eventually influence the balance and distribution of the microflora in soil (Bernal-Vicente et al., 2008). The antagonistic and mutualistic capacities of beneficial bacteria are highly dependent on the dynamics of bacterial

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population in composts, which commonly follow the trend of initial increase, reduction during the thermophilic phase and become stable during the curing phase. The rapid reduction could be attributed to the thermophilic temperature and the communities tend to be stable after consuming the readily available nutrients (Selvam et al., 2012).

Compost, commonly known as a bioagent and biofertilizer, is able to reduce the incidence of plant diseases by suppressing soil-borne pathogens and provide essential plant nutrients (Garcia et al., 2004; Pugliese et al., 2008; Ros et al., 2005; Termorshuizen et al., 2006; Wahyuni et al., 2010). Composts are widely used for promoting long-term productivity of agroecosystem. Their physical, chemical and biological characteristics on regulating phytopathogen were previously investigated by various researchers (EL-Masry et al., 2002; Serra-Wittling et al., 1996). Commonly used compost from green and yard waste, straw, bark, biowaste and municipal sewage sludge had a positive control effect against *Verticillium dahliae* on eggplant, *Rhizoctonia solani* on cauliflower, *Phytophthora nicotianae* on tomato, *Phytophthora cinnamomi* on lupin and *Cylindrocladium spathiphylli* on peace lily (Garcia et al., 2004; Pugliese et al., 2008; Ros et al., 2005; Termorshuizen et al., 2006; Wahyuni et al., 2010).

The dynamics of dominant microbial communities during the transition from mesophilic to thermophilic and to mesophilic again are a function of temperature in the composting mass. Sufficient populations of microorganisms with biocontrol functions in mature composts have a tangible beneficial role in fitting a proper rhizosphere for plant growth. They also have the capacities to mineralize the nutrients through biodegradation. Furthermore, some of these microbes show powerful antipathogenic properties by competing for available nutrients or producing chitinolytic enzymes, siderophores and indole-3-acetic acid (IAA) which destroy the phytopathogens (Wu et al., 2015). Bernal-Vicente et al. (2008) showed that mature citrus compost showed powerful biocontrol effect on *F. oxysporum* and improved the plant yield. The urban organic and yard waste compost was also shown to control *F. oxysporum* efficiently (Pugliese et al., 2008).

Traditional Chinese Medicine (TCM) is an essential part of the healthcare system in China, Hong Kong and in several Asian countries, while considered as a complementary or alternative medical system in most Western countries. Export of medicines amounts to 0.24 million tonnes/year that includes about 0.2 million tonnes of raw herbs which account for about 20% of the annual harvest in China.

The active ingredients of the TCM are often the secondary metabolites of the plants extracted through decoction. Generally the extraction efficiency is around 50% while the residual herbal residue still contain the active ingredient. Recently, the use of these Chinese medicine herbal residues (CMHRs) as a co-composting substrate to compost the food waste was reported with a view to achieve the disposal of both types of wastes (Zhou et al., 2014). However, as some of the active ingredients in the CMHRs are expected to have antimicrobial property, they may affect the bacteria and fungi present in the composting mass. Thus it will influence the microbial community and eventually the composting process. Therefore it is essential to assess the microbial population and often the numerically dominant bacteria and fungi play a significant role in the composting process; thus the knowledge on the dominant microbes will provide insight to develop a compost with multiple-benefits.

Therefore, this study investigated the effects of CMHRs on the bacterial and fungal populations; on the composition of numerically dominant bacteria and fungi; and antagonistic/mutualistic capacities of the dominant bacteria and fungi during food waste–CMHR co-composting. Furthermore, the antipathogenic effect of the compost extracts against two important fungal plant pathogens *A. solani* and *F. oxysporum* was also investigated.

2. Material and methods

2.1. Substrates and composting

The CMHRs were collected daily from the clinic of the School of Chinese Medicine, Hong Kong Baptist University for a month and all samples were stored at 4 °C to minimize decomposition before mixed uniformly to obtain representative samples for subsequent composting process. A synthetic food waste (FW) prepared using rice, bread, cabbage and boiled pork with the fresh weight mixing ratio of 13:10:10:5 (Wong et al., 2009) was used as the food waste substrate. Selected physiochemical properties of the substrates are presented in Table 1. Food waste was co-composted with sawdust and CMHRs at the ratios of 5:5:1 and 1:1:1 on dry weight basis (w/w), and another treatment with food waste mixed with sawdust at 1:1 on dry weight basis (w/w) served as the control. The initial moisture contents of all composting mixtures were adjusted to 55–60% and C/N ratio of ~25. In addition, 2.25% of lime (dry weight basis, w/w) was added to all treatments to alleviate the onset of low pH during the initial stages of FW composting (Wong et al., 2009).

For each treatment, ~7.5 kg of substrate mixture was loaded into a 20-L bench-scale in-vessel compost reactor and composted for 56 days. The reactors were connected to a computer control system with WMA-2 gas analyzer (PP Systems, Herts, UK) and thermosensors for on-line measurement of carbon dioxide and temperature, respectively (Wong et al., 2009). Aeration was provided at 0.5 L/min/kg, based on dry composting mass, using an aerator pump for the first two weeks; then the rate of aeration was reduced to 0.25 L/min/kg. These rates were fixed based on the O_2 requirement for FW composting as reported previously (Wong et al., 2009). Periodically the composting mass in each reactor was mixed before adjusting the moisture content, and about 200 g of samples from each treatment were collected on day 0, 7, 28 and 56 for physiochemical and biological analysis.

2.2. Bacterial and fungal community dynamics during FW–CMHRs composting

2.2.1. Bacterial and fungal populations

A culture independent method was used to quantify the bacterial and fungal populations of the composting mass. Genomic DNA was extracted from 0.3 g fresh compost sample using QIAamp DNA stool mini kit (Qiagen, Hilden) following the manufacturer's recommendations. Extracted DNAs were qualitatively analyzed using agarose (2%) gel electrophoresis and quantified using Nanodrop spectrophotometer; and used as the template for polymerase chain reaction (PCR) amplification.

Table 1
Selected properties of the food waste, sawdust and Chinese medicinal herbal residues (CHMRs) used as the raw materials in the study.

Parameters	Food waste	Sawdust	Chinese medicinal herbal residues	
			Summer	Winter
Moisture content (%)	59.0 (0.02)	7.24 (0.03)	63.0 (0.13)	65.1 (0.16)
Total organic carbon (%)	45.5 (1.70)	52.9 (0.91)	48.0 (0.41)	47.0 (0.48)
Total Kjeldahl nitrogen (%)	3.28 (0.04)	0.59 (0.04)	1.62 (1.32)	1.55 (0.08)
C/N ratio	13.9 (0.35)	89.8 (4.56)	29.6 (2.21)	30.3 (2.67)