

## Article

# The Efficacy of Functional Composts Manufactured Using Spent Coffee Ground, Rice Bran, Biochar, and Functional Microorganisms

Aalfin-Emmanuel Santharajan, Yong-Hun Han and Sung-Cheol Koh \*

Division of Civil, Environmental Engineering and Logistics System, Korea Maritime and Ocean University, Busan 49112, Korea; eaalfi@gmail.com (A.-E.S.); sby81419@naver.com (Y.-H.H.)

\* Correspondence: skoh@kmou.ac.kr; Tel.: +82-10-9900-7294

**Abstract:** Spent coffee grounds (SCGs), one of the world's most discarded wastes, may be an excellent resource as an organic fertilizer because of its richness in nutrients. The objective of this study was to develop a quality functional compost using SCGs, rice bran, biochar, SCG extract, and functional microbes (plant growth promoting and plant pathogen-suppression bacteria), and then to test their functional efficacy for a potential commercial application. Essentially, two types of representative composts (Tr\_1 and Tr\_5 on the laboratory and pilot scale, respectively) were developed and passed all the official commercial quality standards. For pilot-scale composting, populations of *Halotalea uc*, *Corynebacterium nuruki*, and *Lactobacillus acidipiscis* increased by augmentation of the composting microbes (MA-1) and the functional microbes (*Bacillus cereus* SB-3, *Bacillus toyonensis* SB-4, and *Streptomyces sasae* St-3). The higher total flavonoid content (11% increase compared to control) of pepper leaves in PT-1 and the higher TEAC in PT-1 (36.2%) and PT-2 (32.5%) proved the efficacy of the functional composts bioaugmented with the functional microbes. The seedling growth of radish seeds treated with *Streptomyces sasae* St-3 as a biocontrol agent significantly increased despite the presence of the pathogen *Fusarium oxysporum* f. sp. *lactucaae*. The total phenol content and TEAC in pepper plant leaves were significantly higher in Tr\_5 than in the control (Tr\_4), whereas there were no differences in Tr\_4 and Tr\_5 infested with the fungal pathogens, indicating that SB-3, SB-4, and St-3 cultures amended within the compost (Tr\_5) may facilitate the production of the antioxidants in the absence of the pathogens. However, a significant reduction in the antioxidants (total phenolic content and TEAC) was observed in the pepper plants whose roots were infected with the pathogens, indicating that the pathogens could neutralize functionalities of the functional microbes. It was concluded that the enhancement of functional microbes in the compost would aid in the biological control of pathogens in the soil environment. Further functional compost studies are necessary in terms of mechanisms of plant growth-promotion, mechanisms of pathogen suppression by the actinobacterial biocontrol agents, and interactions between the two mechanisms, as well as quality enhancement of the composts.

**Keywords:** spent coffee grounds (SCGs); rice bran; functional compost; antioxidant; plant-growth promoting bacteria; plant pathogen-suppression bacteria



**Citation:** Santharajan, A.-E.; Han, Y.-H.; Koh, S.-C. The Efficacy of Functional Composts Manufactured Using Spent Coffee Ground, Rice Bran, Biochar, and Functional Microorganisms. *Appl. Sci.* **2021**, *11*, 7703. <https://doi.org/10.3390/app11167703>

Academic Editors: Rafael López Núñez and Francisca Suárez Estrella

Received: 27 July 2021

Accepted: 19 August 2021

Published: 21 August 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Compost is considered an environmentally-friendly soil amendment for growing crops because it better builds up more organic matter in the soil than chemical fertilizers [1]. Composting is also an efficient way for organic waste reuse [2]. There is an urgent need to manage these wastes reasonably. In composting, microbes played a vital role in the degradation of organic matter [3]. The degradation products of cellulose can provide energy and carbon sources for the growth and reproduction of microbes [4]. Compost piles usually contain a mixture of carbon-rich and nitrogen-rich materials. Carbon-rich materials, such as woodchips, sawdust, and straw [5], are often shared between commercial composts and backyard composts. The choices for nitrogen-rich materials are based on

accessibility. At the same time, animal manure is more economical and accessible for commercial composting. Such a difference in the selection of nitrogen-rich materials can cause different properties in the final compost products.

Coffee is the second most valuable commodity in the world after oil and its derivatives [6]. However, the processing of coffee cherries, and the milling of dried beans, roasting of green beans, and brewing of coffee contribute to environmental pollution by generating large amounts of bio-waste each year. Production of instant coffee and coffee brewing accounts for approximately 6 million tons of spent coffee grounds (SCGs) per year worldwide [7]. SCGs are usually mixed with common garbage [8,9], causing major environmental issues. Therefore, finding strategies to valorize the utilization of this organic residue is of much interest.

In today's society, the use of SCGs as a soil fertilizer (mineral addition) to promote plant growth is popular [10,11]. An alternative way to use SCGs is their processing into fertilizer without the costly and lengthy process of composting. In this way, organic matter unencumbered by pollution can be a valuable source of nutrients for organic farming, acting in support of the dissemination of the principles of sustainable agriculture. Horticultural plants grow poorly in soils amended with SCGs, likely due to phytotoxic effects [12].

Gomes et al. [13] reported that composting SCGs prior to use as fertilizer reduces toxicity to plants because the amount of phytotoxic compounds including heavy metals, ethylene, and ammonia present in SCGs is reduced. Composting is a common practice for recycling bio-waste and is regarded as a simple and efficient method to convert agricultural industrial wastes into stable, non-toxic, pathogen-free, and nutrient-rich products for soil conditioners and plant fertilizers [14]. The chemical composition of SCGs indicates several applications for this residue. For instance, the presence of nitrogen (about 1.2–2.3%), phosphorus (0.02–0.5%), and potassium (0.35%) [15] suggests its utilization in agriculture as fertilizer or as a soil improver [16]. The contents of elements such as K, Mn, Mg, and Na of lettuce significantly increased when coffee meal was properly mixed with municipal solid waste and composted (5%) [17]. The high-temperature (120–200 °C) and high-pressure water treatment used in this method facilitates the extraction of water-soluble components from SCGs, including proteins, minerals, and organic acids [18]. However, there have been few studies on the application of the water-soluble extracts from SCGs for plant growth.

Rice bran was reported to have a good effect in optimizing the composting process, since it contains some essential material for microbial metabolism [19]. Chang and Chen [20] stated that rice bran was not a bulking agent in the food composting process. Rice bran has been reported to have a good effect to optimize the composting process, since it contains some essential materials for microbial metabolism [19,21]. Rice bran consists of many materials that are essential for microbial growth in organic solid waste composting processes. The use of fresh bran in upland vegetable crops sometimes creates germination inhibition, as well as growth suppression of the crop [22]. However, the influence of these materials on microbial metabolism is not yet known. Considering the above facts, the use of rice bran compost could be a useful way for eco-friendly and non-chemical weed control in organic farming systems of upland vegetables [22].

Biochar is a carbonaceous material obtained from pyrolysis of biomass residues in the absence of oxygen [23]. The physicochemical, chemical, and microbiological properties of a composting pile are favorable for the interaction with biochar, and, consequently, it is expected that a synergy would be established between the pool of organic matter, nutrients, and microbial biomass of the composting material and the physicochemical properties of biochar [24]. The addition of biochar during composting had several benefits: reducing the compost toxicity, the compost exhibiting higher moisture content and lower waste density, N retention during composting, and decreasing potentially pathogenic microorganisms [25]. Co-application of wheat straw (WS) and wheat straw biochar (WSB) with nutrients (MF) at 1% and 2% doses enhanced carbon and nitrogen contents in soil (i.e., their water soluble fractions such as DOC and DON) [26]. The synergistic effect of biochar and MF triggered the increase in the population density and activity of soil microorganisms.

The typical pathogens found in the composting of municipal solids waste and sewage sludge can be viruses, bacteria, protozoa, or helminths. They are usually heat-sensitive. The heat generated during the composting process can eliminate them, leading to a pathogen-free end compost product, and can be defined as a disinfection or sanitization process [27]. In an in-vessel composting, *Salmonella* sp., *Staphylococcus aureus*, and *Shigella* sp. were removed, while the coliform counts were reduced by a 2-log scale after 40 days of composting [28]. Antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) were significantly removed by 22.8–99.7% during the thermophilic phase of composting facilitated by the compound microbial inoculants carrying *Bacillus* sp. and yeasts [29]. This composting was helpful to remove the pathogen from the final compost product.

Plant growth-promoting bacteria (PGPB) produce a wide variety of molecules, which improve plant growth and productivity. These PGPB increased the production of phytohormones or other molecules that protect plants from biotic and abiotic stress, increased mineral nutrition, modulating ethylene levels in plants and the production of volatile organic compounds [30]. Furthermore, PGPB act as a potential symbiont and degrades the xenobiotic compounds to protect the plants. Inoculation of PGPB along with a mixture of organic amendments and biochar could be an effective way to overcome the problem of heavy metal toxicity [31]. There have so far been few studies, to the best of our knowledge, regarding functional composts carrying PGPB and plant pathogen-inhibiting microbes.

In this study, we aimed to manufacture and commercialize eco-friendly and high-quality functional composts using spent coffee grounds (and their extracts), biochar, rice bran, and functional microorganisms (plant growth-promoting bacteria and plant pathogen-inhibiting actinomycetes). We also tried to evaluate functional efficacies of the composts in terms of commercial application quality, microbial community structures, plant-growth promotion, antioxidant production in crops, and plant pathogen inhibition.

## 2. Materials and Methods

### 2.1. Isolation and Selection of Plant Growth-Promoting Bacteria and Plant Pathogen Inhibiting Bacteria, and Their Growth Medium Preparation from Spent Coffee Grounds

The target strains for this study were *Bacillus* sp. (plant-growth-promotion function) and *Actinomyces* sp. (plant-pathogen-suppression function) that were isolated from forest soils at the Korea Maritime and Ocean University (Busan, Korea) to identify microorganisms and register a patent strain. The media for *Bacillus* sp. and *Actinomyces* sp. were trypticase soy agar (TSA, Difco) and International Streptomyces Project (ISP-4), respectively. To establish a proper medium for the functional microorganisms, three kinds of extractions of coffee beans were tried: (1) spent coffee grounds suspended in distilled water (10%, w/w) and heat-treated (121 °C for 15 min); (2) spent coffee grounds suspended in distilled water (10%, w/w) and extracted in a shaking incubator (150 rpm) at 25 °C for 1 h; and (3) spent coffee grounds suspended in 80% ethanol (10%, w/w) and extracted in a shaking incubator (150 rpm) at 25 °C for 1 h. Each medium was tested to grow the potential functional microbial isolates (SB-3, SB-4, and St-3). The growth status (density and contamination degree) was monitored by plating the cultures onto TSA (SB-3 and SB-4) and ISP (St-3) for 4 days. The best medium was chosen based on the highest growth status. The potential plant-growth-promoting bacteria were selected based on radish germination activity and its seedling growth activity after growing 20 radish seeds on sterilized filter paper soaked with 2 mL of the bacterial culture grown on the best SCG extraction medium for 3 days. The plant-pathogen-suppressing bacteria (*Actinomyces* sp.) were selected based on the growth inhibition effect of the bacteria (on ISP medium 4 agar plates) against known plant pathogens (*Fusarium oxysporum* f. sp. *lycopersici* KACC 40032, *Fusarium oxysporum* f. sp. *lactucae* KACC 42795, *Pythium ultimum* KACC 40705, and *Pythium* sp. KACC 40581). The final selected isolates were identified using the 16S rRNA gene analysis system at the Institute of Microbial Ecology and Resources, Mokwon University, Daejeon, Korea.

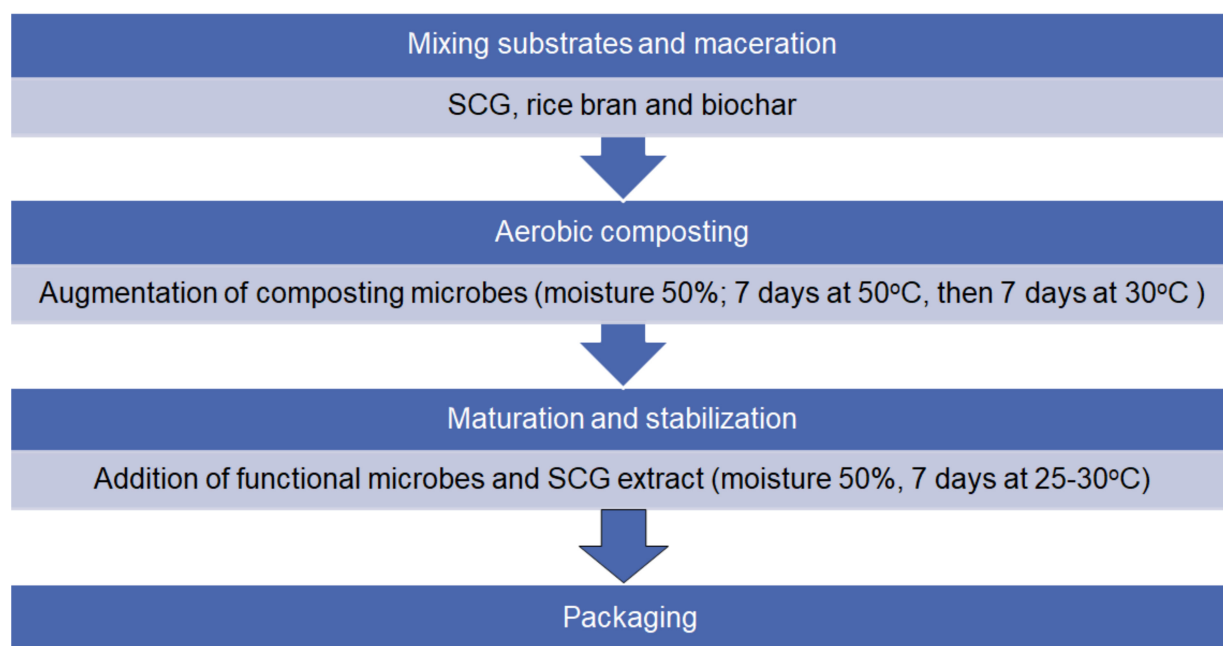
## 2.2. Procedures for Composting Using Spent Coffee Grounds, Rice Bran, and Biochar, and Phytotoxicity Test for the Manufactured Composts

The substrates used for composting were spent coffee grounds, rice bran, and biochar. The spent coffee grounds were collected from several local coffee shops in Yeongdo-gu, Busan, Korea, and dried in a shaded place to 40–50% moisture. Recipes for substrates and microbial inoculum used for the composting at the laboratory and pilot scales are shown in Table 1, and the composting process is shown in Figure 1. The percentages of spent coffee grounds, rice bran, and biochar in the mixtures were 81.5% (*w/w*), 15.8%, and 2.5%, respectively, on the laboratory scale, and 49.07%, 49.28%, and 1.51%, respectively, on the pilot scale. The aerobic composting was performed by augmenting the microbial agent (MA-1) [15] as a facilitator for the composting, and the augmentation ratios were 0.2% (*w/w*) on the laboratory scale and 0.13% on the pilot scale. Each mixture was maintained under 50% moisture content at 50 °C for 7 days. Afterward, each compost was matured and stabilized by adding functional microbes (plant-growth-promoting bacteria and plant-pathogen-inhibiting bacteria) grown on SCG extract (10%, *w/w*) for 5 days. The moisture content (50%) and temperature (25–30 °C) were maintained for 7 days in the process.

**Table 1.** Recipes for substrate and microbial inoculum used for composting on the laboratory and pilot scales.

Code	Unit	Location of Substrates	Mixture of Substrates and Composting Microbes				Inoculation of Functional Microbes			Composting Location
			SPG	Defatted Rice Bran	Biochar	Microbial Inoculation (MA-1)	Total (kg)	<i>Bacillus</i> sp. (SB-3 and SB-4)	<i>Streptomyces sasae</i> (St-3)	
NC	% kg	Lab	81.5 1.46	16.0 0.283	2.5 0.045	0 0	1.7	0 0	0 0	Lab
Tr_1	% kg	Lab	81.5 1.46	16.0 0.283	2.5 0.045	0.2 0.004	1.7	1 1.7 mL	1 1.7 mL	Lab
Tr_2	% kg	Factory	47 25.85	50 27.25	3 1.65	0.2 0.11	55	0 0	0 0	Lab
Tr_3	% kg	Factory	47 25.85	50 27.25	3 1.65	0.2 0.11	55	1 1.5 L	1 1.5 L	Lab
Tr_4	% kg	Factory	47 25.85	50 27.25	3 1.65	0.2 0.11	55	1 1.5 L	1 1.5 L	Factory
Tr_5	% kg	Factory	47 25.85	50 27.25	3 1.65	0.2 0.11	55	1 1.5 L	1 1.5 L	Factory

Two types of composting vessels were used on the laboratory scale: round plastic bucket type (depth × height = 23 × 29 cm) and rectangular plastic trays (length × width × height = 70 × 50 × 20 cm), all without a heat control system. The complete compost was dried to 40–50% moisture and kept at cool room temperature until the subsequent experiments. Pilot composting was performed using a mixing and fermentation system for feedstuff (Model DDK-802F; 1120 mm (W) × 2030 mm (H) × 2240 mm (L); Daedongtech, Inc., Seoul, Korea; working volume 1300 L; automatic temperature control and mixing functions). Quality evaluation analysis of the manufactured composts was performed by AT Analysis Center Co., Ltd. (Incheon, Korea) according to the criteria required by the Office of Rural Development, Seoul, Korea. The phytotoxicity test for the composts was performed based upon the germination index of the test plants. Seed germination rate (GR) and root elongation (RE) were measured and calculated using the formula  $GI = GR \times RE / 100$ , where GI is the germination index, GR is the germination rate, and RE indicates root elongation [32].



**Figure 1.** Manufacturing process of the functional composts using SCGs, rice bran, biochar, and functional microbes.

### 2.3. Microbial Community Analysis of the Manufactured Complete Composts

The microbial community structures in the composts were analyzed using 16S rRNA gene-based pyrosequencing, since various microbial communities were involved in the composting process. The detailed analysis procedures were described by Kim et al. (2014) [33]. In brief, the total bacterial genomic DNA of each compost was isolated using a PowerSoil<sup>®</sup> DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). Variable regions (V1–V3) of the bacterial 16S rRNA gene were then amplified from the genomic DNA, and the library construction, sequencing, and all subsequent analyses were performed using a 454 GS FLX Junior Sequencing System (Roche, Branford, CT, USA) and the accompanying protocols. Statistical analyses of microbial communities were accomplished with the Mothur program, using a 3% difference cut-off value (Schloss et al., 2009). Principal coordinate analysis (PCoA) and fast Unifrac analysis were conducted using CLcommunity software (Chunlab, Inc., Seoul, Korea).

### 2.4. Crop Growth Conditions for Test of the Manufactured Composts

As a part of the efficacy test of the complete composts, the effects of the composts on the growth of commercial crops such as pepper were studied on the laboratory scale (Table 2). The growth test was performed using a round plastic pot (upper diameter 15 cm, lower diameter 10 cm, and height 12 cm) carrying universal potting soil composed of peat (Gramoflor GmbH, KG, Vechta, Germany) (1.2 kg) under ambient indoor conditions (25 °C; 300–500 lux sunlight). Commercial organic fertilizer (Coffee Microbial Fertilizer, KT Trade Inc., Seoul, Korea) and chemical fertilizer (All Purpose Compound Fertilizer, Nousbo Inc., Suwon, Korea; 100 g/m<sup>2</sup>) were amended according to the manufacturer's recommendations for the duration of the experiment, whereas the manufactured compost was amended once in the beginning of the experiment. Each amendment was conducted in triplicate pots, each of which was planted with three seedlings of pepper, and the growth experiment lasted 2 weeks. The growth effect was examined for the three representative plants in each pot, and leaf length and leaf width of the second branch from the crown top were measured weekly for 17 days, and perfectly grown leaves were taken for the analysis of antioxidant production.



**Table 2.** Conditions for pepper plants for the test of growth and antioxidant production by treatment of functional compost in pots.

Treatment Code	Kinds of Compost or Fertilizer	SB Cultures (% <i>, w/w</i> ) *	St-3 Culture (% <i>, w/w</i> ) **	Compost or Fertilizer Amount (g)
PT_1C	NC	0	0	24
PT_1	Tr_1	1.0	1.0	24
PT_2C	Tr_2	0	0	24
PT_2	Tr_3	1.0	1.0	24
PT_3C	Tr_4	0	0	24
PT_3	Tr_5	1.0	1.0	24
PT_4	Commercial compost product	-	-	9.0
PT_5	Chemical fertilizer	-	-	1.54

\* *Bacillus cereus* SB-3 KCTC14418BP and *Bacillus toyonensis* SB-4 KCTC14417BP; \*\* *Streptomyces sasae* St-3.

### 2.5. Analysis of Antioxidants from Leaves of Pepper Plants Grown on the Composts

To determine DPPH radical scavenging activity, 1 g of dried pepper leaves and fruits was used to extract and prepare a sample solution for the antioxidant test. The assay of 1,1-diphenyl 2-picryl-hydrazyl (DPPH) radical scavenging activity was performed following the methods in a previous report [34]. Briefly, 1 mL of ethanol extract and 5 mL of a freshly prepared DPPH ethanol solution (0.1 mM) were mixed thoroughly and kept in the dark. After 30 min of incubation at ambient temperature, the absorbance was read against a blank at 517 nm by using a UV–visible spectrophotometer (Model POP, Optizen, Inc., Seoul, Korea). The percentage of free radical scavenging activity was calculated as follows: scavenging activity (%) =  $(1 - (A_{517 \text{ nm of sample}} / A_{517 \text{ nm of blank}})) \times 100$ .

The total phenolic content as an antioxidant material was determined by the Folin–Ciocalteu method using gallic acid as the standard following the methods in a previous report [34]. The extract was prepared from 1 g of dried and macerated leaf or fruit with the incubation time of 24 h and filtered. The ethanol extract solution was shaken for 1 min with 0.4 mL of Folin–Ciocalteu reagent (1 M) and mixed with 0.8 mL of Na<sub>2</sub> CO<sub>3</sub> (20%, *w/v*). After 8 min of incubation, the mixture was centrifuged at 15,000 × *g* for 10 min. The absorbance of the supernatant was measured at 730 nm using a spectrophotometer. The results are expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g) based on the standard curve for gallic acid concentration versus absorbance (*R* = 0.873) [34].

The total flavonoid content was determined following the method of Park et al. (2008) [35]. In a 10 mL test tube, 0.3 mL of extract, 3.4 mL of 30% methanol, 0.15 mL of NaNO<sub>2</sub> (0.5 M), and 0.15 mL of AlCl<sub>3</sub>·6H<sub>2</sub>O (0.3 M) were mixed. After 5 min, 1 mL of NaOH (1 M) was added. The solution was mixed well, and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was obtained using rutin standard solution (0 to 100 mg/L) under the same procedure described earlier. The total flavonoids were expressed as milligrams of rutin equivalents per gram of dried fraction.

For TEAC analysis, the ABTS reagent was prepared by mixing equal amounts of aqueous 7.4 mM ABTS and 2.6 mM potassium persulfate solutions, which were allowed to react overnight in the dark [36]. Trolox standard solution was prepared, and absorbance was recorded on the UV–visible spectrophotometer (Model POP, Optizen Inc., Seoul, Korea) at 730 nm.

### 2.6. Inhibition Effects of *Streptomyces sasae* St-3 on Root Rot Plant Pathogens in Petri Dishes and Pots

The inhibitory effect of the isolated strain St-3 on phytopathogens *Pythium ultimum* (KACC 40705) and *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) was analyzed based on radish germination activity and its seedling growth activity (Table 3). The experiment was accomplished by growing 20 radish seeds on a sterilized filter paper soaked with 2 mL of compost extract of Tr<sub>4</sub> within a petri dish in which *Streptomyces sasae* St-3 and the pathogen cultures were inoculated.

**Table 3.** Experimental conditions for determining the inhibition effect of *Streptomyces sasae* St-3 on plant pathogens against radish germination in the presence of the functional compost extract using petri dishes.

Treatment Code	Treatment	Compost Extract	Inoculation of <i>Streptomyces sasae</i> * St-3	Root Rotting Pathogen **
CPP	Control	Tr_4	–	<i>Pythium ultimum</i>
TPP	Treatment	Tr_4	+	<i>Pythium ultimum</i>
CPF	Control	Tr_4	–	<i>Fusarium oxysporum</i> f. <i>lactucae</i>
TPF	Treatment	Tr_4	+	<i>Fusarium oxysporum</i> f. <i>lactucae</i>

\* Grown on SCG extraction solution (10%, w/w; 121 °C for 15 min); \*\* grown in ISP-4 medium.

The inhibitory effect of strain St-3 on the same phytopathogens was also analyzed using pots carrying 1.2 kg of universal potting soil composed of peat (Gramoflor GmbH, KG, Vechta, Germany) and 24 g of the manufactured compost (Tr\_4). Each pot carried 4 pepper plants infected with the root rot plant pathogens *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) and *Pythium ultimum* (KACC 40705) (Table 4).

**Table 4.** Effects of the potential plant growth-promoting bacteria (*Bacillus cereus* SB-3 and *Bacillus toyonensis* SB-4) and the pathogen-inhibiting bacterium (*Streptomyces sasae* St-3) on pepper plants infected with the root rot plant pathogens *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) and *Pythium ultimum* (KACC 40705) in the pot soil.

Treatment Code	Compost Amended	Inoculation of St-3, SB-3, and SB-4 *	Root Rotting Pathogen **
Tr_4	Tr_4	+	–
Tr_5	Tr_5	+	–
Tr_4_PU	Tr_4	–	<i>Pythium ultimum</i>
Tr_5_PU	Tr_5	+	<i>Pythium ultimum</i>
Tr_4_FO	Tr_4	–	<i>Fusarium oxysporum</i> f. <i>lactucae</i>
Tr_5_FO	Tr_5	+	<i>Fusarium oxysporum</i> f. <i>lactucae</i>

\* Grown on SCG extraction solution (10%, w/w; 121 °C for 15 min); \*\* grown on ISP-1 medium.

## 2.7. Statistical Analysis

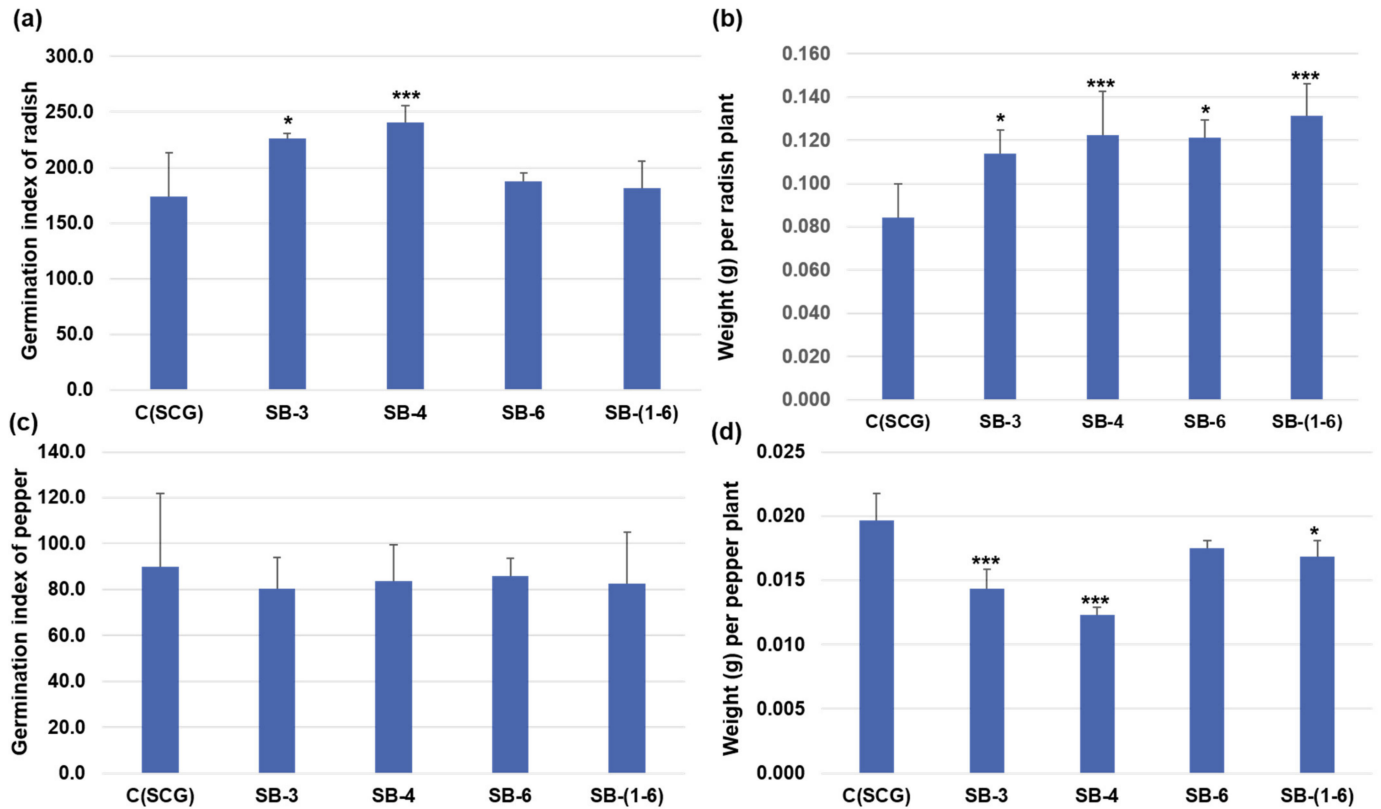
The data from the experiments were subjected to analysis of variance for a completely random design using SPSS statistical software [37]. The data are presented as the mean  $\pm$  standard deviation of duplicate or triplicate determinations according to the test and treatment. Comparison of means was analyzed by a t-test calculated in MS Excel and Duncan's test using the SPSS system (IBM SPSS statistics 19), and differences were considered significant when  $p$  was  $<0.05$ ,  $<0.01$ , or  $<0.001$ .

## 3. Results

### 3.1. Isolation and Selection of Plant Growth-Promoting Bacteria and Plant Pathogen-Inhibiting Bacteria, and Their Growth Medium Preparation from Spent Coffee Ground

Six *Bacillus* sp. strains (SB-1, SB-2, SB-3, SB-4, SB-5, and SB-6) and one strain of actinomycetes (St-3) were isolated from the forest soils at the Korea Maritime and Ocean University. The *Bacillus* sp. strains were tested for their potential plant-growth-promoting function, and the actinomycetes strain was tested for its suppression of plant pathogens (Figures 2 and 3). For the experiment, the bacteria were inoculated and selected according to the previous result. The selected bacteria, SB-3, SB-4, SB-6, and SB (1-6) (mixed), in SCG extract, and SB-3 and SB-4 showed high rates for radish (Figure 2a,b), while the growth of pepper was relatively inhibited under the same experimental conditions (Figure 2d). This appeared to be due to the sensitivity of pepper seedlings to the SCG extraction medium compared to the radish medium. Strain St-3 was observed to effectively inhibit the growth of the root rot fungi such as *Fusarium oxysporum* f. sp. *lycopersici* (KACC 40032) (Figure 3a), *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) (Figure 3b), *Pythium ultimum* (KACC 40705) (Figure 3c), and *Pythium* sp. (KACC 40581) (Figure 3d). Microbial identification was

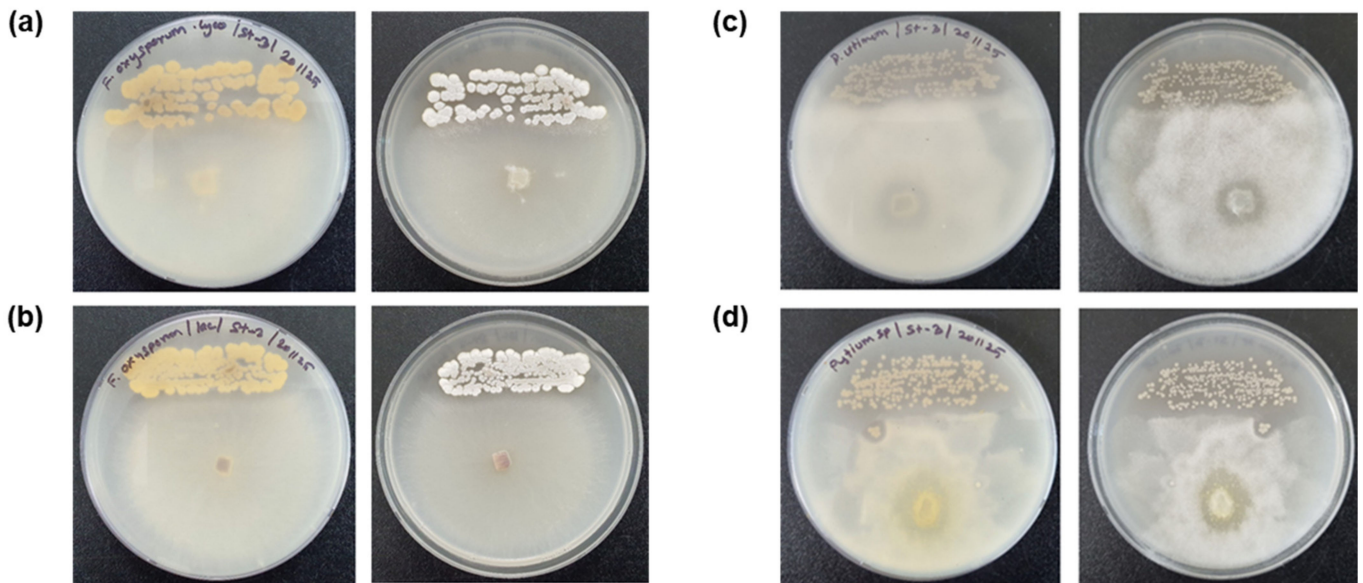
performed for the finally selected three strains (SB-3, SB-4, and St-3) based on 16S rDNA sequencing analysis and were identified as *Bacillus cereus* SB-3 (KCTC14418BP), *Bacillus toyonensis* SB-4 (KCTC14417BP), and *Streptomyces sasae* St-3 (KCTC14416BP), respectively.



**Figure 2.** Effects of potential plant growth-promoting bacteria grown on SCG extract on the growth of radish plant (a,b) and pepper plant (c,d). SB-(1-6) indicates the mixed culture of strains SB-1, SB-2, SB-3, SB-4, SB-5, and SB-6. \* indicates the significance of the treatment comparing with the control; \*— $p < 0.05$ ; \*\*\*— $p < 0.001$ .

The phytopathogens *Fusarium oxysporum* f. sp. *lycopersici* (KACC 40032), *Fusarium oxysporum* f. sp. *lactucaae* (KACC 42795), *Pythium ultimum* (KACC 40705), and *Pythium* sp. (KACC 40581) were highly suppressed by *Streptomyces sasae* St-3 in three days of incubation at 30 °C (Figure 3). The growth and the colony size of St-3 was unique between the two different inoculated pathogens. The fungi were spread throughout the plates on day one, and St-3 dominated with its growth, which suppressed the entire part where the St-3 was streaked. The St-3 culture was analyzed and confirmed under a microscope. The bacteria were separately cultured and stored at 4 °C for further analyses.





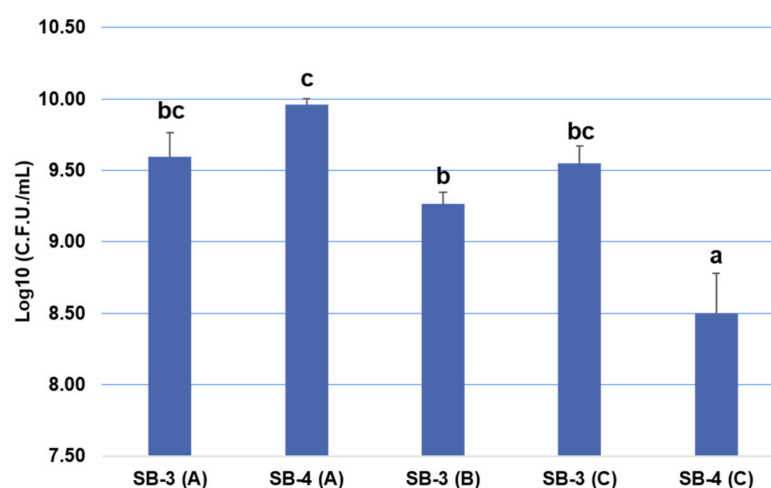
**Figure 3.** Analysis of the inhibitory effect of *Streptomyces sasae* St-3 on phytopathogens (*Fusarium oxysporum* f. sp. *lycopersici* (KACC 40032) (a), *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) (b), *Pythium ultimum* (KACC 40705) (c), and *Pythium* sp. (KACC 40581) (d)).

### 3.2. Impacts of the Various SCG Extracts on the Growth of Potential Plant Growth-Promoting Bacteria

The potential plant growth promoting bacteria (SB-3 and SB-4) selected from phytotoxicity analysis were tested to find their growth status in the three different media (Figure 4). The extraction of SCGs using distilled water by heat treatment at 121 °C for 15 min showed the highest CFU/mL of each culture of both bacteria. The SGC media extracted with 80% ethanol at room temperature produced considerable growth of SB-3 with a log<sub>10</sub> CFU/mL of 9.72, and a lower CFU/mL for SB-4. SB-3 (A) and SB-4 (A) showed higher population densities (log<sub>10</sub> CFU/mL = 9.72 and 9.99, respectively) compared to those grown in other extracts. The extraction of SCGs using distilled water by a shanking incubator (150 rpm) at 25 °C for one hour produced a somewhat high CFU/mL with SB-3, whereas SB-4 was hardly observed in the plates due to indigenous bacterial contamination from the spent coffee grounds. Therefore, SCG extraction (10%, w/w in water) by heat treatment (121 °C for 15 min) may be commercially applicable to culturing plant-growth-promoting bacteria.

### 3.3. Composting Process Monitoring, Physico-Chemical Analysis, and Maturity Quality Test of the Manufactured Composts

The temperature and moisture content were monitored for the composting treatments. A total of 1.2 kg of substrate mixture (NC and Tr\_1) and 5 kg (Tr\_2, Tr\_3, Tr\_4, and Tr\_5) were composted, where the highest temperature reached was 55 °C, which then decreased to 30–36 °C. The temperatures were maintained during the whole 14 days of composting. The moisture content was adjusted every two days to 50–55% due to the high temperature. The quality control data of the functional composts manufactured are shown in Table 5. The representative composts (Tr\_1 and Tr\_5) met all the commercial quality standards approved by the Office of Rural Development of the South Korean government. Tr\_5 carried higher contents of organic matter, total phosphorus, and total potassium than Tr\_1, probably due to the significantly higher content of rice bran in Tr\_5.



**Figure 4.** Effects of various SCG extracts on the growth of potential plant growth-promoting bacteria. (A) SCG in distilled water (10%, w/w) and extracted by heat treatment (121 °C for 15 min); (B) SCG in distilled water (10%, w/w) and extracted by a shaking incubator (150 rpm) at 25 °C for 1 h; (C) SCG suspended in 80% ethanol (10%, w/w) and extracted by a shaking incubator (150 rpm) at 25 °C for 1 h. Letters above the error bar indicates the comparison of samples using Duncan's test.

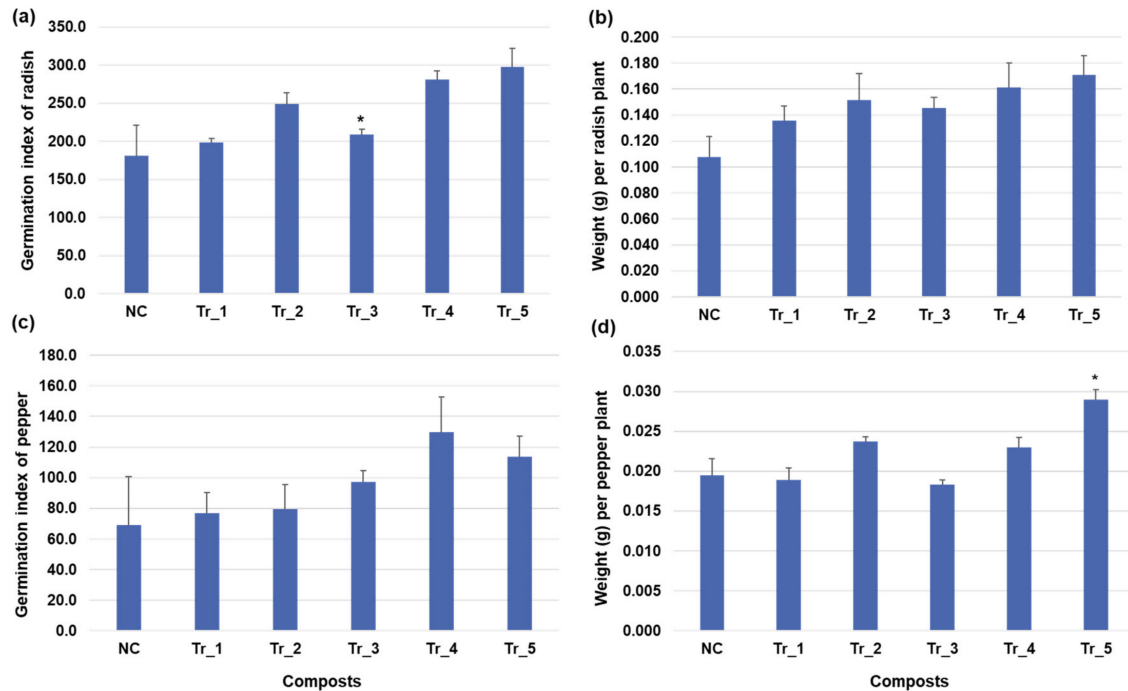
**Table 5.** Quality control data of the functional composts manufactured on the laboratory and pilot scales using SCGs, rice bran, biochar, and functional microbes \*.

Evaluation Criteria	Unit	Commercial Quality Standard	Tr-1	Tr-5
Organic matter/N ratio		<45	19.68	26
NaCl	%	<2.0	0.051	0.042
Moisture	%	<55	45.08	29.08
As	mg kg <sup>-1</sup>	<45	ND	ND
Cd	mg kg <sup>-1</sup>	<5	ND	ND
Hg	mg kg <sup>-1</sup>	<2	ND	ND
Pb	mg kg <sup>-1</sup>	<130	0.2	0.04
Cr	mg kg <sup>-1</sup>	<200	2.35	1.75
Cu	mg kg <sup>-1</sup>	<360	43.8	16.27
Ni	mg kg <sup>-1</sup>	<45	2.36	1.05
Zn	mg kg <sup>-1</sup>	<900	49.3	61.99
Organic matter	%	>30	49.6	62.68
<i>E. coli</i> O157:H7	ND	ND	ND	ND
Salmonella	ND	ND	ND	ND
Maturity	Instrument analysis (CoMMe-100) **	Complete humification	Complete humification	Complete humification
HCl insoluble material	%	< 25	0.34	0.27
Total N (%) ***		-	2.52	2.41
Total P (%) ***		-	1.22	3.36
Total K (%) ***		-	0.89	1.66

\* The analysis was performed by AT Analysis Center Co., Ltd., Incheon, Korea, and was officially approved by the Office of Rural Development, a part of the South Korean government; ND, no detection; \*\* Soiltek, Inc., Jeju, Korea; \*\*\* not included in the official evaluation criteria.

To analyze the physio-chemical properties and maturity quality, we performed a phytotoxicity test. The composts were diluted ten times, and the germination index and the weight of the grown plants were monitored using radish and pepper plants. The germination index of Tr\_5 was 49% higher than the NC and 5.6% higher than Tr\_4. The addition of SB cultures and St-3 might be the reason for the enhancement in GI in Tr\_5. The bioaugmented Tr\_5 was higher in radish plant weight by 58%, and 48% higher in pepper

plant weight compared to NC. Tr\_3 was significantly higher than the NC in both plants. Tr\_4 dominated in pepper plant by 88% and was 10.9% higher than the NC and Tr\_5. Even though the ratio of Tr\_2 and Tr\_3 was similar to that of Tr\_4 and Tr\_5, composting in the laboratory (Tr\_2 and Tr\_3) and pilot (Tr\_4 and Tr\_5) scales was clearly differentiated by the higher values for pilot-scale composting (Figure 5).



**Figure 5.** Effects of the functional composts on the growth of radish plants (a,b) and pepper plants (c,d). NC, Tr\_2, and Tr\_4 were the controls for Tr\_1, Tr\_3, and Tr\_5, respectively; \* indicates the significance of the treatment comparing with the control; \*— $p < 0.05$ .

### 3.4. Microbial Community Analysis of the Manufactured Composts

The microbial community analysis was compared between the control and the treatments on the laboratory and pilot scales. The integrated samples Tr\_2 and Tr\_3 were excluded due to their low effectiveness. *Sphingobacteriaceae\_uc* and *B. multivorans* were highly dominant in both NC and Tr\_1. The *B. coagulans* group, *B. thermoamylovorans* group, and *O. oleidegradans* were comparatively higher in Tr\_1 with 19% of the distribution. Most of the dominant species were lower in Tr\_1 compared to the NC, except the *B. coagulans* group because of the addition of SB and St-3 cultures (Figure 6a). *Halotalea\_uc* was dominant in both Tr\_4 and Tr\_5. *P. agglomerans* and *P. septica* were dominant in Tr\_4, and *C. nuruki* and *L. acidipiscis* in Tr\_5. *Halotalea\_uc* was the dominant species commonly present in all the samples, covering 40–50% of the distribution in Tr\_4 and Tr\_5 (Figure 6b). Even though we applied the same ingredients for the laboratory and pilot scales, the distribution of the microbial communities differed. The ratio of the substrates used in the composting and its processing environment might be the reason for the dominance of different microorganisms.

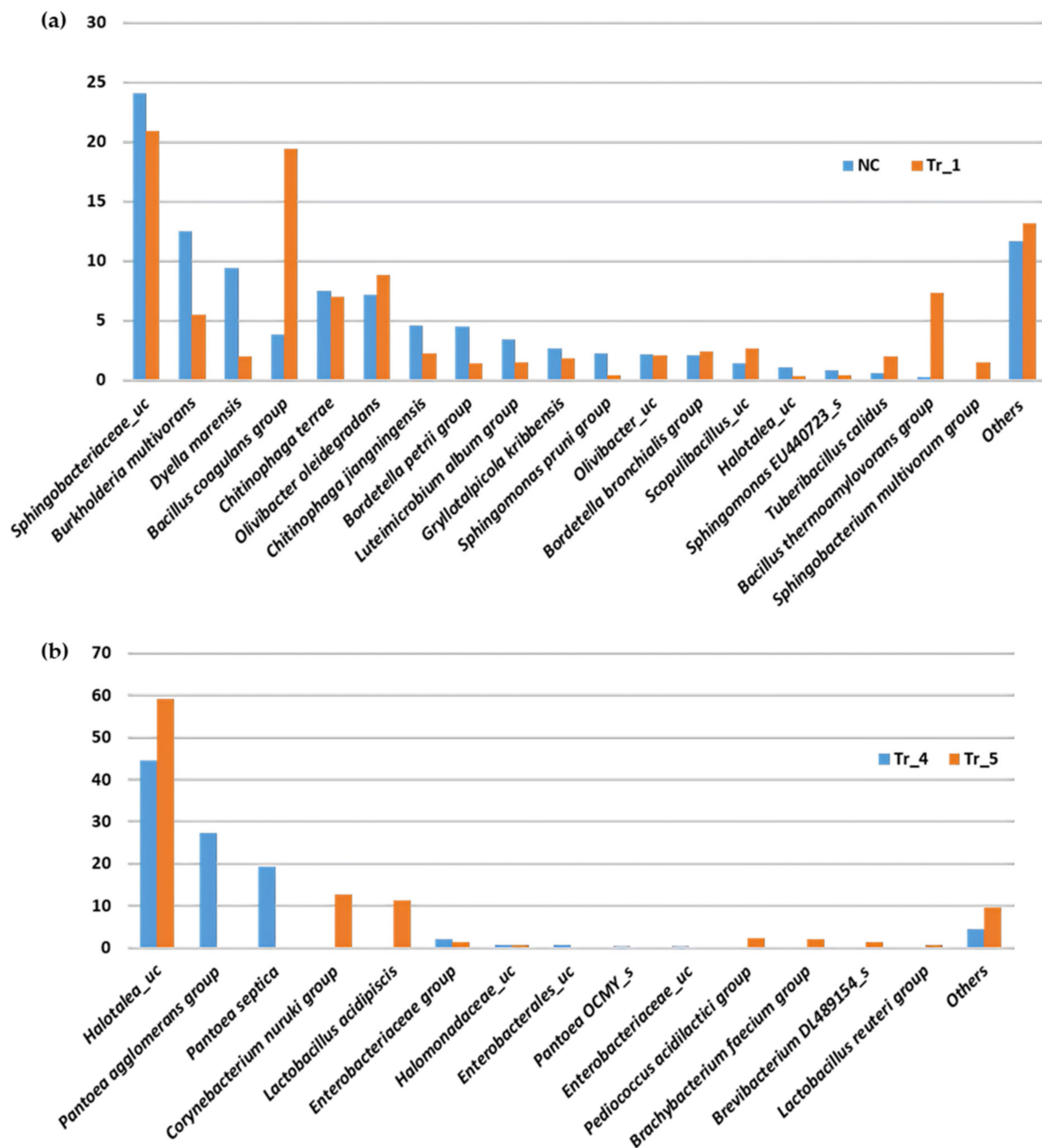
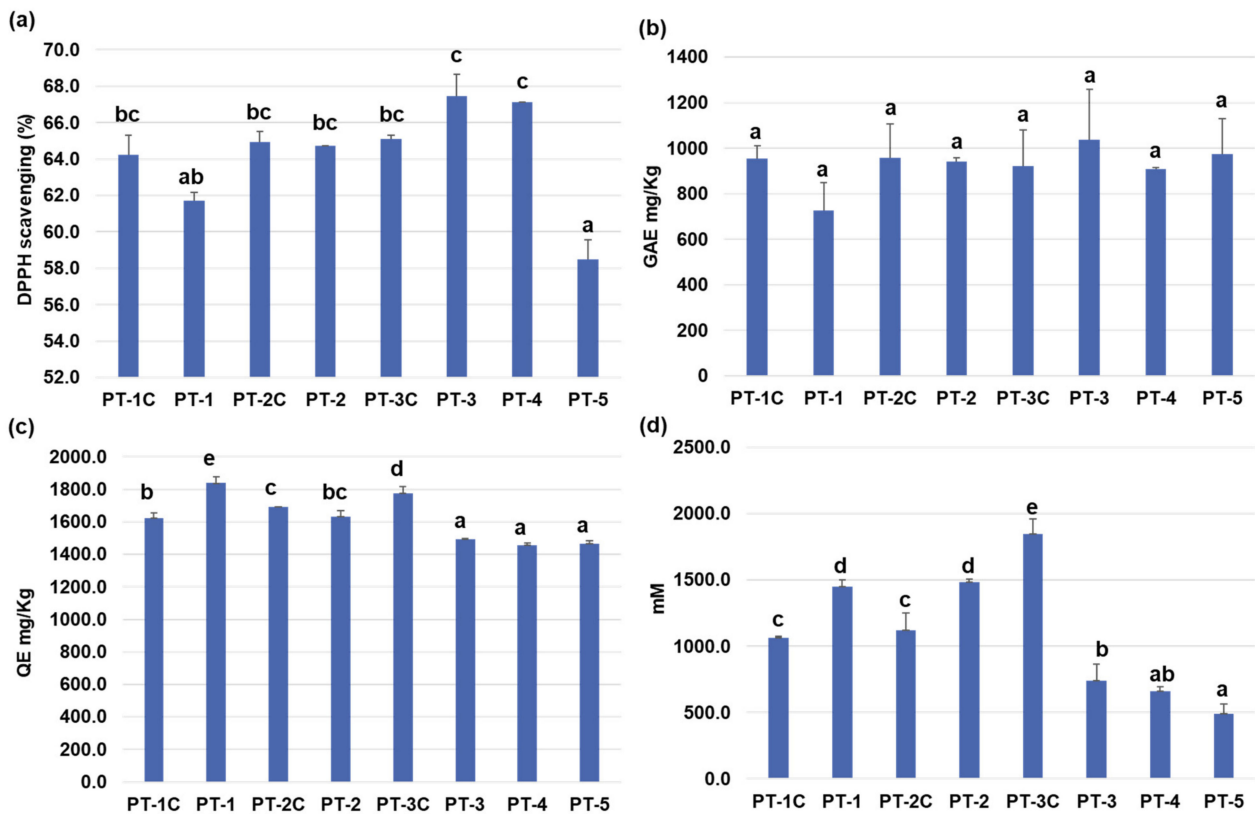


Figure 6. Microbial community structures of the representative functional composts. (a) Laboratory scale; (b) Pilot scale.

### 3.5. Analysis of Pepper Growth and Antioxidant Production by Treatment with Functional Composts in Pot Experiments

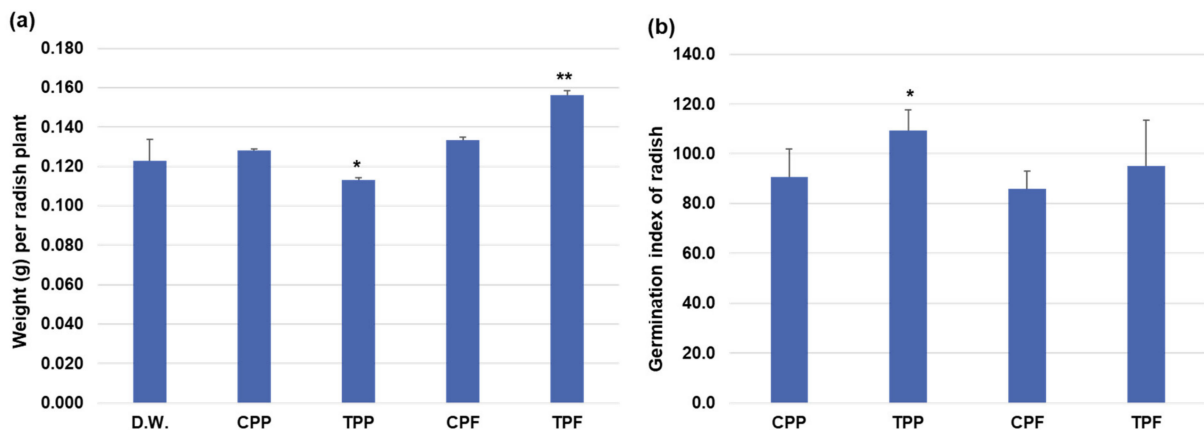
To perform the antioxidant tests, the pepper leaves of each treatment were dried, crushed, and extracted using 80% ethanol. The antioxidant properties were examined with three controls of different composts with three treatments including commercial organic and chemical fertilizer (PT-4 and PT-5) (Figure 7). There were generally no significant differences among the composting samples in terms of DPPH and TPC (Figure 7a,b). PT-1 was higher in TFC by 13.1% (Figure 7c), whereas PT-1 and PT-2 were higher in TEAC than their controls (PT-1C and PT-2C) by 36.2% and 32.5%, respectively (Figure 7d).



**Figure 7.** Antioxidant activity of leaf extracts of pepper plants grown in soil amended with SCG composts, commercial compost, and chemical fertilizer. The tests included (a) DPPH scavenging activity, (b) total phenolic content (TPC), (c) total flavanoid content (TFC), and (d) Trolox equivalent antioxidant activity (TEAC). Letters above the error bar indicates the comparison of samples using Duncan's test.

### 3.6. Inhibition Effect of *Streptomyces Sasae St-3* on Plant Pathogens during Radish Germination in the Presence of Functional Compost Extract

The treatment with St-3 to the radish seeds infested with the fungal pathogens (*P. ultimum* and *F. oxysporum*) was compared to the control (infestation of the pathogens without St-3). The radish seedling growth in TPF was significantly higher than its control, CPF, by 12% (Figure 8a). However, the radish germination index in TPP was significantly higher compared to CPP as the control (by 20%) (Figure 8b).

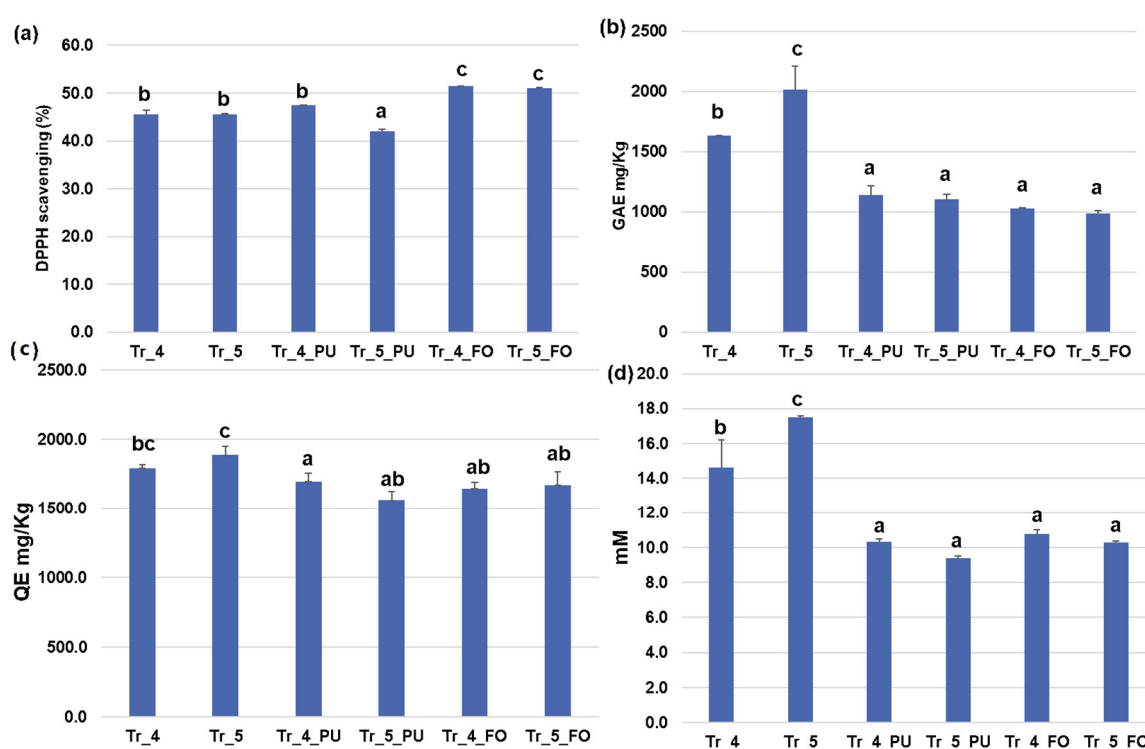


**Figure 8.** Inhibition effects of *Streptomyces sasae St-3* on the root rot plant pathogens *Pythium ultimum* (KACC 40705) (TPP) and *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) (TPF), whose controls were CPP and TPP, respectively; (a) Weight per radish plant, (b) Germination index of radish plant; \*  $p < 0.05$  and \*\*  $p < 0.01$ .



### 3.7. Effects of the Potential Plant Growth-Promoting Bacteria and Plant Pathogen-Inhibiting Bacterium on Pepper Plants Infected with Root Rot Plant Pathogens

The antioxidant activity of leaf extracts of pepper plants grown in soil amended with the functional SCG composts carrying *Streptomyces sasae* St-3 and infected with root rot plant pathogens *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) (FO) and *Pythium ultimum* (KACC 40705) (PU) was determined (Figure 9). The total phenol content and TEAC in pepper plant leaves were significantly higher in Tr\_5 than in the control (Tr\_4) by 23.5% and 19.5%, respectively, but there were no differences amongst all the treatments in the presence of the fungal pathogens (Figure 9b,d). Compared to the pathogen-treated samples (Tr\_5-PU and Tr\_5-FO), Tr\_5 showed higher TPC, TFC, and TEAC activity (12–48%). Moreover, we observed no differences in the total flavanoid content between Tr\_4 and Tr\_5 in the presence and absence of the fungal pathogens, whereas DPPH scavenging was significantly higher in the presence of the pathogen (*Fusarium oxysporum* f. sp. *lactucae*) (Figure 9c).



**Figure 9.** Antioxidant activity of leaf extracts of pepper plants grown in soil amended with the functional SCG composts carrying *Streptomyces sasae* St-3 and infected with root rot plant pathogens *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) (FO) and *Pythium ultimum* (KACC 40705) (PU). The tests included (a) DPPH scavenging activity, (b) total phenolic content (TPC), (c) total flavanoid content (TFC), and (d) Trolox equivalent antioxidant activity (TEAC). Letters above the error bar indicates the comparison of samples using Duncan's test.

## 4. Discussion

The germination index (GI) was used to evaluate the phytotoxic substances present in the compost, which is one of the most sensitive parameters that accounts for the low toxicity affecting root growth [38]. The *Bacillus* sp. strains were tested for their potential plant-growth-promoting function, and SB-3 and SB-4 were selected for the composting process. *Bacillus* sp. is known to enhance the availability of macro- and micronutrients in the soil and their uptake by host plants [39]. Enhancement in the maximum proximate chemical constituents including carbohydrate, proteins, dry matter, etc., was recorded by the application of *Bacillus* sp. Stefan et al. [39] identified the significant role of *Bacillus* isolates in the enhancement in essential amino-acid contents and other nutritive chemical constituents in food crops [40]. The seed germination and plant growth of radish and

pepper plants are significantly influenced by the nutrients available in the soil. The results of the two different plants confirms the difference between the several potential microbes tested in this process (Figure 2). Plants absorb phosphorus (P) and nitrogen (N) from the soil through root transporters, but the bioavailable forms of P and N are limited in rhizospheres [41]. *Bacillus* sp. convert the complex form of essential nutrients, such as P and N, to a simple available form that is used during uptake by plant roots [42,43]. Phosphate is involved in nucleic acid, phospholipid, and adenosine triphosphate (ATP) metabolism, among other metabolic pathways, in plant cells [44]. The secretion of phosphatases and organic acids from *Bacillus* sp. acidifies the surrounding environment to facilitate the conversion of inorganic phosphate into free phosphate [42], [45]. In this study, *Bacillus cereus* SB-3 (KCTC14418BP) and *Bacillus toyonensis* SB-4 (KCTC14417BP) appeared to promote the growth of radish. The genomic analysis of plant-associated *B. cereus* 905 showed that the genes related to plant-growth-promoting traits were highly conserved [46]. *B. cereus* SA1 inoculation increased the biomass, chlorophyll content, and chlorophyll fluorescence of soybean plants under normal and heat stress conditions. The SA1 strain can be used as a good temperature-tolerant candidate for the mitigation of heat stress damage in soybean plants and can be commercialized as a biofertilizer [45]. *Bacillus toyonensis* COPE52 was reported to produce indoleacetic acid and protease activity, and emit volatiles such as acetoin, 2,3-butanediol, and dimethyl disulfide as potential plant-growth-promoting mechanisms, leading to growth in biomass and chlorophyll content in blueberry plants [47]. *B. toyonensis* COPE52 also showed plant-growth-promoting activities including indole-3-acetic acid (IAA), protease activity, and biofilm formation, as well as antifungal activity against *Botrytis cinerea*, even in saline conditions [48].

Analysis of the inhibitory effect of *Streptomyces sasae* St-3 on the plant pathogenic fungi revealed its potential antifungal activity (Figure 3). This acidophilic characteristic of *Streptomyces* sp. may help it to adapt to the soil conditions. Moreover, a recent study found that the antifungal activity of *Streptomyces* inhibits fungi including *Fusarium oxysporum*, *Pythium aphanidermatum*, *P. ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Sclerotinia minor*, and *S. sclerotiorum*, and other soil-borne plant pathogens [49]. We also demonstrated that the growth of phytopathogens *Fusarium oxysporum* f. sp. *lycopersici* (KACC 40032), *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795), *Pythium ultimum* (KACC 40705), and *Pythium* sp. (KACC 40581) were highly suppressed by *Streptomyces sasae* St-3 after three days of incubation at 30 °C. This confirms that St-3 is a potential candidate for targeted anti-fungal treatment for crops.

The in vitro antibiosis of *Streptomyces violaceusniger* G10 against *Fusarium oxysporum* f. sp. *cubense* race 4 was demonstrated by inhibition zones formed in the cross-plug assay plates [50]. The in vitro antagonistic effects against the pathogen appeared to be caused by the antifungal metabolites produced by strain G10 in liquid media, leading to swelling, distortion, and excessive branching of hyphae and inhibition of spore germination. The chitinase enzyme produced by *Streptomyces griseorubens* E44G might play a role as an antifungal agent against *F. oxysporum* f. sp. *lycopersici*, digesting the fungal cell wall composed of chitin [51]. *Streptomyces sasae* TG01 can inhibit the growth of phytopathogenic fungi *Fusarium solani* and *Fusarium oxysporum* by producing secondary metabolites including 2-methyl-1,3-dioxolane as the major constituent [52]. The two most effective bioactive root symbiont *Streptomyces* strains (H2 and H3) were selected based on in vitro petri plate seedling bioassays (IPSB), effectively controlling the damping-off disease caused by *Pythium aphanidermatum* as well as behaving as plant growth-promoting rhizobacteria able to increase the fresh and dry weight of tomato plants [53].

The use of SCGs extracts to grow beneficial bacteria may allow a circumvention of this by-product disposal, producing a negative environmental impact as well as resulting in the loss of a rich source of nutrients and bioactive compounds [54]. Recent research proved that *Bacillus* fermented SCGs extracts exhibited increased phenolic- and flavonoid-related antioxidant activity [54]. A recent article reported that the GI of wheat seeds grown in the extract of the vermicomposted SCG (10%, w/w) showed 205% [55]. In this study

the *Bacillus* sp. strains (SB-3 and SB-4) grown on the SCGs extracts (10%, w/w) showed higher germination indices (GIs) (231% and 246%) (Figure 2a). This non-cytotoxic effect by coffee-derived extracts was correlated with the coffee roasting level and the presence of chlorogenic acids and melanoidins [56].

The moisture content of all the composts was adjusted to 50–55% to maintain sufficient microbial activity for composting. Temperature, which is one of the key indicators of composting, determines the rate of many biological processes and plays a significant role in the evolution and succession of microbiological communities [57]. Rice bran addition in Tr\_4 and Tr\_5 (50%, w/w) generally enhanced the germination index and the growth of radish and pepper. Islam et al. [58] reported that the GI of radish seeds for compost with the chicken manure was 130%. The GI of radish seeds for Tr\_5 in this study was much higher (297%). The n-hexane, acetone, and water-soluble fractions obtained from rice bran were more effective on seed germination [59]. The addition of biochar during the composting process provided some benefits by helping to retain useful nutrients in the biochar. Biochar can also favor the creation of humic acids over fulvic acids during the composting of the rice bran and can alleviate nitrogen loss in the mature composting products. The addition of biochar during composting reduced the time required to enter the thermophilic phase and helped generate a higher temperature and longer duration in the thermophilic stage [60].

At laboratory-scale composting, the *Bacillus coagulans* group, *Bacillus thermoamylovorans* group, and *Olivibacter oleidegradans* became dominant after the addition of functional microbes (strains SB-3, SB-4, and St-3). *Bacillus coagulans* and *Bacillus thermoamylovorans* were dominant in the thermophilic stage of composting and had a greater capacity to degrade less biodegradable, complex organic compounds such as lignocelluloses [61]. *Olivibacter oleidegradans* was isolated from a biofilter clean-up facility on a hydrocarbon-contaminated site, whereas *Olivibacter composti* sp. nov. was isolated from compost collected at a greenhouse (shih). At pilot-scale composting, the growth of *Halotalea\_uc*, *Corynebacterium nuruki*, and *Lactobacillus acidipiscis* was facilitated by the addition of the functional microbes. Previous reports have mentioned that *Sphingobacteriaceae* and *Halotalea* were dominant species in functional compost [62]. *Corynebacterium nuruki* was isolated from an alcohol fermentation starter, which was a mixture of grains and various micro-organisms including mold, yeast, and bacteria (Shin). There was a significant dominance of members of the *Lactobacillus* genus (*L. brevis*, *L. plantarum*, *L. oris*, and *L. johnsonii*) and *Lactobacillus acidipiscis* as a minor species [63]. It appeared that *Corynebacterium nuruki* and *Lactobacillus acidipiscis* originated from the rice bran used for composting materials in this study. Recent findings proved that *B. coagulans* might produce specific enzymes such as nitrile hydratase, endoinulinase, and alkaline lipase used for electrotransformation and fermentation for composting [64]. *Lactobacillus acidipiscis* was reported as a compost bacterium to be linked with nitrogen metabolism and fermentation in compost [65].

PT-1 had a significantly higher TFC, whereas PT-1 and PT-2 demonstrated a higher level of TEAC compared to each control (Figure 7c,d). This indicated that inoculation with the functional microbes (SB-3, SB-4, and St-3) during the stabilization stage of the composting process can enhance the antioxidant components in pepper plants. DPPH and TPC showed much higher activity with the range of 60–70% of SCV and 700–1000 GAE mg/kg compared to the previous compost augmented with PGPB [66]. The high QE of PT-1 in the total flavonoid content and PT-1 and PT-2 in TEAC proved the dominance of antioxidant activity through the bioaugmented compost (Figure 7c,d). Flavonoids have hydroxyl groups that are functional in promoting free radical scavenging activity and ABTS chelation of metal ions, hence mediating antioxidant activities [67]. It was hypothesized that plant growth-promoting bacteria (PGPB) facilitate the production of non-enzymatic antioxidants and increase the availability of mineral nutrients. It was reported that PGPB also release/increase the availability of mineral elements such Cu, Fe, Mn, Zn, etc., to plants by chelation and acidification of soil [57]. Siderophore production may be a major feature of PGPB. It is essential for certain iron–sulfur complex enzymes and iron-containing proteins

and plays a major role in plant growth by participating in the synthesis of chlorophyll. Evidence also shows that beneficial microbes can enhance plants' tolerance to adverse environmental conditions [68]. For example, due to the application of a potassium-releasing strain of *Bacillus edaphicus*, enhanced plant growth and potassium uptake were reported under nutrient deficiency and heavy metal contamination in cotton and rapeseed [69]. Similarly, in this study, radish and pepper plants treated with the SB-3, SB-4, and St-3 cultures showed higher growth rates and increased antioxidant activity. The higher germination indices of TPP and TPF, and the higher growth rate of TPF indicated that treatment with SB-3, SB-4, and St-3 cultures was effective in stimulating radish growth in the presence of plant pathogens (*Pythium ultimum* and *Fusarium oxysporum* f. sp. *lactucae*) (Figure 8). Research showed that biological control agents including *S. sasae* can produce hydrolytic enzymes, such as protease, glucanases, amylase, and chitinase, to destroy the components of the fungal cell wall, which is an important mechanism involved in the biocontrol of phytopathogenic fungi [70].

The total phenol content and TEAC activity in pepper plant leaves were significantly higher in Tr\_5 than in the control (Tr\_4) but we found no differences in all the treatments in the presence of fungal pathogens (Figure 9b,d), indicating that amendment with SB-3, SB-4, and St-3 cultures can facilitate the production of antioxidants in the absence of the pathogens. Moreover, we found no differences in DPPH scavenging or total flavonoid content between Tr\_4 and Tr\_5 in the presence and absence of the fungal pathogens, whereas DPPH scavenging significantly increased in the presence of the pathogen *Fusarium oxysporum* f. sp. *lactucae* (Figure 9a,c). The antioxidant mode of action was reported in many host–pathogen interactions. For example, many oxidative enzymes such as peroxidase, catalase, ascorbate oxidase, and polyphenol oxidase were detected as a result of infection with many pathogens [71]. In our study, the significant reduction in antioxidants (total phenolic content and TEAC) in Tr\_4 PU, Tr\_4 FO, Tr\_5 PU, and Tr\_5 FO (Figure 9b,d) might be due to inhibition by the fungal pathogens producing phenolic, benzoic, ferulic, coumaric, and protocatechoic acid [72]. In this study, amendment with SB-3, SB-4, and St-3 cultures did not seem to counteract the pathogen's functioning. Actinobacteria (i.e., *Streptomyces* sp.) combined with other plant-growth-promoting bacteria (i.e., *Bacillus* sp.) should be effective for the biocontrol of pathogens, plant growth promotion, and positive interaction with many plants [73].

## 5. Conclusions

The higher total flavonoid content in PT-1 and the higher TEAC activity in PT-1 and PT-2 proved the efficacy of the functional composts bioaugmented with functional microbes. The seedling growth of radish seeds treated with *Streptomyces sasae* St-3 as a biocontrol agent significantly increased even in the presence of the pathogen *Fusarium oxysporum* f. sp. *lactucae*. The total phenol content and TEAC in pepper plant leaves were significantly higher in Tr\_5 than in the control (Tr\_4), whereas there were no differences in Tr\_4 and Tr\_5 infested with the fungal pathogens, indicating that SB-3, SB-4, and St-3 cultures amended within the compost (Tr\_5) may facilitate the production of the antioxidants in the absence of the pathogens. However, a significant reduction in the antioxidants (total phenolic content and TEAC) was observed in the pepper plants whose roots were infected with the pathogens, indicating that the pathogens could neutralize functionalities of the functional microbes. It was concluded that a good establishment of the functional microbes within the composts would contribute to the biocontrol of the pathogens in the soil environment. Further functional compost research is necessary on issues such as mechanisms of plant-growth promotion (production of plant growth regulators, enhanced iron availability by production of siderophores, nitrogen fixation, and phosphate solubilization), mechanisms of plant-pathogen suppression by actinobacterial biocontrol agents (antibiotics, lytic enzymes, hyperparasitism, and competition), and the interaction between the two mechanisms in addition to quality enhancement of the composts.



## 6. Patents

There is a patent resulting from the work reported in this manuscript: “Functional composts carrying spent coffee ground extracts and their manufacturing methods”, Korea Patent No. 10-2021-0009108).

**Author Contributions:** Conceptualization, S.-C.K.; methodology, A.-E.S. and Y.-H.H.; software, A.-E.S.; investigation, A.-E.S. and S.-C.K.; writing—original draft preparation, A.-E.S.; writing—review and editing, S.-C.K.; visualization, S.-C.K.; supervision, S.-C.K.; project administration, Y.-H.H.; funding acquisition, S.-C.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the LINC+ Project 2020 grant sponsored by Ministry of Education and National Research Foundation, Korea (No. 2020-E-G055-010124).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

## References

- Evanylo, G.; Sherony, C.; Spargo, J.; Starner, D.; Brosius, M.; Haering, K. Soil and water environmental effects of fertilizer-, manure-, and compost-based fertility practices in an organic vegetable cropping system. *Agric. Ecosyst. Environ.* **2008**, *127*, 50–58. [[CrossRef](#)]
- Lakhdar, A.; Scelza, R.; ben Achiba, W.; Scotti, R.; Rao, M.A.; Jedidi, N.; Abdelly, C.; Gianfreda, L. Effect of municipal solid waste compost and sewage sludge on enzymatic activities and wheat yield in a clayey-loamy soil. *Soil Sci.* **2011**, *176*, 15–21. [[CrossRef](#)]
- Zhang, M.; He, L.-Y.; Liu, Y.-S.; Zhao, J.-L.; Zhang, J.-N.; Chen, J.; Zhang, Q.-Q.; Ying, G.-G. Variation of antibiotic resistome during commercial livestock manure composting. *Environ. Int.* **2020**, *136*, 105458. [[CrossRef](#)]
- Nascimento, F.X.; Rossi, M.J.; Glick, B.R. Ethylene and 1-Aminocyclopropane-1-carboxylate (ACC) in Plant–Bacterial Interactions. *Front. Plant Sci.* **2018**, *9*, 114. [[CrossRef](#)]
- Silva, C.F.; Azevedo, R.S.; Braga, C.; Da Silva, R.; Dias, E.S.; Schwan, R.F. Microbial diversity in a bagasse-based compost prepared for the production of *Agaricus brasiliensis*. *Braz. J. Microbiol.* **2009**, *40*, 590–600. [[CrossRef](#)]
- Tucker, C.M. *Coffee Culture*, 2nd ed.; Anthropology of Stuff Series; Routledge: New York, NY, USA, 2018.
- Getachew, A.T.; Chun, B.S. Influence of pretreatment and modifiers on subcritical water liquefaction of spent coffee grounds: A green waste valorization approach. *J. Clean. Prod.* **2017**, *142*, 3719–3727. [[CrossRef](#)]
- Cruz, R.; Mendes, E.; Torrinha, Á.; Morais, S.; Pereira, J.A.; Baptista, P.; Casal, S. Revalorization of spent coffee residues by a direct agronomic approach. *Food Res. Int.* **2015**, *73*, 190–196. [[CrossRef](#)]
- Cruz, R.; Gomes, T.; Ferreira, A.; Mendes, E.; Baptista, P.; Cunha, S.; Pereira, J.A.; Ramalhosa, E.; Casal, S. Antioxidant activity and bioactive compounds of lettuce improved by espresso coffee residues. *Food Chem.* **2014**, *145*, 95–101. [[CrossRef](#)] [[PubMed](#)]
- Liu, K.; Price, G.W. Evaluation of three composting systems for the management of spent coffee grounds. *Bioresour. Technol.* **2011**, *102*, 7966–7974. [[CrossRef](#)] [[PubMed](#)]
- Ribeiro, J.P.; Vicente, E.; Gomes, A.P.D.; Nunes, M.I.; Alves, C.; Tarelho, L.A.C. Effect of industrial and domestic ash from biomass combustion, and spent coffee grounds, on soil fertility and plant growth: Experiments at field conditions. *Environ. Sci. Pollut. Res.* **2017**, *24*, 15270–15277. [[CrossRef](#)]
- Hardgrove, S.J.; Livesley, S.J. Applying spent coffee grounds directly to urban agriculture soils greatly reduces plant growth. *Urban For. Urban Green.* **2016**, *18*, 1–8. [[CrossRef](#)]
- Gomes, T.; Pereira, J.; Ramalhosa, E.; Casal, S.; Baptista, P. Effect of Fresh and Composted Spent Coffee Grounds on Lettuce Growth, Photosynthetic Pigments and Mineral Composition. *VII Congreso Ibérico de Agroingeniería y Ciencias Hortícolas*; SECH e SEAgIng. Available online: <http://hdl.handle.net/10198/8719> (accessed on 21 June 2021).
- Ros, M.; Pascual, J.; Garcia, C.; Hernandez, T.; Insam, H. Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. *Soil Biol. Biochem.* **2006**, *38*, 3443–3452. [[CrossRef](#)]
- Mussatto, S.I.; Carneiro, L.M.; Silva, J.P.A.; Roberto, I.C.; Teixeira, J.A. A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydr. Polym.* **2011**, *83*, 368–374. [[CrossRef](#)]
- Kondamudi, N.; Mohapatra, S.K.; Misra, M. Spent coffee grounds as a versatile source of green energy. *J. Agric. Food Chem.* **2008**, *56*, 11757–11760. [[CrossRef](#)] [[PubMed](#)]
- Cruz, R.; Morais, S.; Mendes, E.; Pereira, J.A.; Baptista, P.; Casal, S. Improvement of vegetables elemental quality by espresso coffee residues. *Food Chem.* **2014**, *148*, 294–299. [[CrossRef](#)] [[PubMed](#)]



18. Zuorro, A.; Lavecchia, R. Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. *J. Clean. Prod.* **2012**, *34*, 49–56. [[CrossRef](#)]
19. Budihardjo, M.A.; Sutrisno, E.; Fatimah, M. Leaves composting process and the influence of rumen content and bran addition. *MATEC Web Conf.* **2018**, *159*, 2033. [[CrossRef](#)]
20. Chang, J.I.; Chen, Y. Effects of bulking agents on food waste composting. *Bioresour. Technol.* **2010**, *101*, 5917–5924. [[CrossRef](#)] [[PubMed](#)]
21. Moongngarm, A.; Daomukda, N.; Khumpika, S. Chemical compositions, phytochemicals, and antioxidant capacity of rice bran, rice bran layer, and rice germ. *APCBEE Procedia* **2012**, *2*, 73–79. [[CrossRef](#)]
22. Khan, M.A.I.; Ueno, K.; Horimoto, S.; Komai, F.; Tanaka, K.; Ono, Y. Evaluation of the use of rice bran compost for eco-friendly weed control in organic farming systems. *Am. J. Environ. Sci.* **2007**, *3*, 235–240. [[CrossRef](#)]
23. Wang, S.; Zhao, X.; Xing, G.; Yang, L. Large-scale biochar production from crop residue: A new idea and the biogas-energy pyrolysis system. *BioResources* **2012**, *8*, 8–11. [[CrossRef](#)]
24. Sanchez-Monedero, M.; Cayuela, M.L.; Roig, A.; Jindo, K.; Mondini, C.; Bolan, N. Role of biochar as an additive in organic waste composting. *Bioresour. Technol.* **2018**, *247*, 1155–1164. [[CrossRef](#)] [[PubMed](#)]
25. Malinowski, M.; Wolny-Koładka, K.; Vaverková, M. Effect of biochar addition on the OFMSW composting process under real conditions. *Waste Manag.* **2019**, *84*, 364–372. [[CrossRef](#)] [[PubMed](#)]
26. Mierzwa-Hersztek, M.; Wolny-Koładka, K.; Gondek, K.; Gałazka, A.; Gawryjolek, K. Effect of coapplication of biochar and nutrients on microbiocenotic composition, dehydrogenase activity index and chemical properties of sandy soil. *Waste Biomass Valorization* **2020**, *11*, 3911–3923. [[CrossRef](#)]
27. Deportes, I.; Guyod, B.; Zmirou, D.; Bouvier, M.C. Microbial disinfection capacity of municipal solid waste (MSW) composting. *J. Appl. Microbiol.* **1998**, *85*, 238–246. [[CrossRef](#)]
28. Fatunla, K.; Inam, E.; Essien, J.; Dan, E.; Odon, A.; Kang, S.; Semple, K.T. Influence of composting and thermal processing on the survival of microbial pathogens and nutritional status of Nigeria sewage sludge. *Int. J. Recycl. Org. Waste Agric.* **2017**, *6*, 301–310. [[CrossRef](#)]
29. Li, K.; Cao, R.; Mo, S.; Yao, R.; Ren, Z.; Wu, J. Swine manure composting with compound microbial inoculants: Removal of antibiotic resistance genes and their associations with microbial community. *Front. Microbiol.* **2020**, *11*, 2686. [[CrossRef](#)]
30. Danish, S.; Zafar-Ul-Hye, M.; Mohsin, F.; Hussain, M. ACC-deaminase producing plant growth promoting rhizobacteria and biochar mitigate adverse effects of drought stress on maize growth. *PLoS ONE* **2020**, *15*, e0230615. [[CrossRef](#)]
31. Danish, S.; Zafar-Ul-Hye, M. Combined role of ACC deaminase producing bacteria and biochar on cereals productivity under drought. *Phyton* **2020**, *89*, 217–227. [[CrossRef](#)]
32. Emmanuel, S.A.; Yoo, J.; Kim, E.-J.; Chang, J.-S.; Park, Y.-I.; Koh, S.-C. Development of functional composts using spent coffee grounds, poultry manure and biochar through microbial bioaugmentation. *J. Environ. Sci. Health Part B* **2017**, *52*, 802–811. [[CrossRef](#)]
33. Kim, I.-S.; Ekpeghere, K.I.; Ha, S.-Y.; Kim, B.-S.; Song, B.; Kim, J.-T.; Kim, H.-G.; Koh, S.-C. Full-scale biological treatment of tannery wastewater using the novel microbial consortium BM-S-1. *J. Environ. Sci. Health Part A* **2014**, *49*, 355–364. [[CrossRef](#)] [[PubMed](#)]
34. Chang, T.-C.; Jang, H.-D.; Lin, W.-D.; Duan, P.-F. Antioxidant and antimicrobial activities of commercial rice wine extracts of Taiwanese *Allium fistulosum*. *Food Chem.* **2016**, *190*, 724–729. [[CrossRef](#)] [[PubMed](#)]
35. Park, Y.-S.; Jung, S.-T.; Kang, S.-G.; Heo, B.G.; Arancibia-Avila, P.; Toledo-Montiel, F.; Drzewiecki, J.; Namiesnik, J.; Gorinstein, S. Antioxidants and proteins in ethylene-treated kiwifruits. *Food Chem.* **2008**, *107*, 640–648. [[CrossRef](#)]
36. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D.H. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* **2006**, *19*, 669–675. [[CrossRef](#)]
37. Jothy, S.L.; Zakaria, Z.; Chen, Y.; Lau, Y.L.; Latha, L.Y.; Sasidharan, S. Acute oral toxicity of methanolic seed extract of cassia fistula in mice. *Molecules* **2011**, *16*, 5268–5282. [[CrossRef](#)]
38. Gu, W.; Zhang, F.; Xu, P.; Tang, S.; Xie, K.; Huang, X.; Huang, Q. Effects of sulphur and *Thiobacillus thioparus* on cow manure aerobic composting. *Bioresour. Technol.* **2011**, *102*, 6529–6535. [[CrossRef](#)]
39. Stefan, V.; Van Herpen, E.; Tudoran, A.A.; Lähteenmäki, L. Avoiding food waste by Romanian consumers: The importance of planning and shopping routines. *Food Qual. Prefer.* **2013**, *28*, 375–381. [[CrossRef](#)]
40. Chitra, S.; Bargavi, P.; Balasubramaniam, M.; Chandran, R.R.; Balakumar, S. Impact of copper on in-vitro biomineralization, drug release efficacy and antimicrobial properties of bioactive glasses. *Mater. Sci. Eng. C* **2020**, *109*, 110598. [[CrossRef](#)]
41. Bidondo, L.F.; Pergola, M.; Silvani, V.; Colombo, R.; Bompadre, J.; Godeas, A. Continuous and long-term monoxenic culture of the arbuscular mycorrhizal fungus *Gigaspora decipiens* in root organ culture. *Fungal Biol.* **2012**, *116*, 729–735. [[CrossRef](#)]
42. Kang, S.-M.; Radhakrishnan, R.; Lee, I.-J. *Bacillus amyloliquefaciens* subsp. *Plantarum* GR53, a potent biocontrol agent resists *Rhizoctonia* disease on Chinese cabbage through hormonal and antioxidants regulation. *World J. Microbiol. Biotechnol.* **2015**, *31*, 1517–1527. [[CrossRef](#)]
43. Kuan, K.B.; Othman, R.; Rahim, K.A.; Shamsuddin, Z.H. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. *PLoS ONE* **2016**, *11*, e0152478. [[CrossRef](#)]

44. Theodorou, M.E.; Plaxton, W. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. *Plant Physiol.* **1993**, *101*, 339–344. [[CrossRef](#)] [[PubMed](#)]
45. Kang, S.-M.; Radhakrishnan, R.; Khan, A.L.; Kim, M.-J.; Park, J.-M.; Kim, B.-R.; Shin, D.-H.; Lee, I.-J. Gibberellin secreting rhizobacterium, *Pseudomonas putida* H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant Physiol. Biochem.* **2014**, *84*, 115–124. [[CrossRef](#)]
46. Zeng, Q.; Xie, J.; Li, Y.; Gao, T.; Xu, C.; Wang, Q. Comparative genomic and functional analyses of four sequenced *Bacillus cereus* genomes reveal conservation of genes relevant to plant-growth-promoting traits. *Sci. Rep.* **2018**, *8*, 17009. [[CrossRef](#)] [[PubMed](#)]
47. Pérez, G.C.; González, C.G.Z. Practices and conceptions of feedback in initial teacher training. *Educ. Pesqui.* **2019**, *45*, e192953. [[CrossRef](#)]
48. Rojas-Solis, D.; Vences-Guzmán, M.Á.; Sohlenkamp, C.; Santoyo, G. Antifungal and plant growth-promoting bacillus under saline stress modify their membrane composition. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 1549–1559. [[CrossRef](#)]
49. Lyu, A.; Liu, H.; Che, H.; Yang, L.; Zhang, J.; Wu, M.; Chen, W.; Li, G. Reveromycins A and B from *Streptomyces* sp. 3–10: Antifungal activity against plant pathogenic fungi in vitro and in a strawberry food model system. *Front. Microbiol.* **2017**, *8*, 550. [[CrossRef](#)]
50. Getha, K.; Vikineswary, S. Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. cubense race 4: Indirect evidence for the role of antibiosis in the antagonistic process. *J. Ind. Microbiol. Biotechnol.* **2002**, *28*, 303–310. [[CrossRef](#)] [[PubMed](#)]
51. Al-Askar, A.A.; Baka, Z.A.; Rashad, Y.M.; Ghoneem, K.M.; Abdulkhair, W.M.; Hafez, E.E.; Shabana, Y.M. Evaluation of *Streptomyces griseorubens* E44G for the biocontrol of *Fusarium oxysporum* f. sp. lycopersici: Ultrastructural and cytochemical investigations. *Ann. Microbiol.* **2015**, *65*, 1815–1824. [[CrossRef](#)]
52. Sudiana, I.M.; Putri, A.; Napitupulu, T.P.; Purnaningsih, I.; Kanti, A. Growth inhibition of *Fusarium solani* and *F. oxysporum* by *Streptomyces sasae* TG01, and its ability to solubilize insoluble phosphate. *Biodiversitas J. Biol. Divers.* **2020**, *21*. [[CrossRef](#)]
53. Hassanisaadi, M.; Bonjar, G.S.; Hosseinipour, A.; Abdolshahi, R.; Barka, E.A.; Saadoun, I. Biological control of *Pythium aphanidermatum*, the causal agent of tomato root rot by two *Streptomyces* root symbionts. *Agronomy* **2021**, *11*, 846. [[CrossRef](#)]
54. Medina, J.J.R.; Ramírez, K.; Rangel-Peraza, J.G.; Bustos-Terrones, Y.A. Increase of content and bioactivity of total phenolic compounds from spent coffee grounds through solid state fermentation by *Bacillus clausii*. *J. Food Sci. Technol.* **2018**, *55*, 915–923. [[CrossRef](#)]
55. González-Moreno, M.; Gracianteparaluceta, B.G.; Sádaba, S.M.; Urdin, J.Z.; Domínguez, E.R.; Ezcurdia, M.P.; Meneses, A.S. Feasibility of vermicomposting of spent coffee grounds and silverskin from coffee industries: A laboratory study. *Agronomy* **2020**, *10*, 1125. [[CrossRef](#)]
56. Priftis, A.; Mitsiou, D.; Halabalaki, M.; Ntasi, G.; Stagos, D.; Skaltsounis, L.A.; Kouretas, D. Roasting has a distinct effect on the antimutagenic activity of coffee varieties. *Mutat. Res. Toxicol. Environ. Mutagen.* **2018**, *829–830*, 33–42. [[CrossRef](#)]
57. Etesami, H.; Hosseini, H.M.; Alikhani, H.A. Bacterial biosynthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, a useful trait to elongation and endophytic colonization of the roots of rice under constant flooded conditions. *Physiol. Mol. Biol. Plants* **2014**, *20*, 425–434. [[CrossRef](#)]
58. Islam, M.; Kasim, S.; Alam, K.; Amin, A.; Hun, T.G.; Haque, M. Changes in chemical properties of banana pseudostem, mushroom media waste, and chicken manure through the co-composting process. *Sustainability* **2021**, *13*, 8458. [[CrossRef](#)]
59. Khan, M.A.I.; Ueno, K.; Horimoto, S.; Komai, F.; Tanaka, K.; Ono, Y. Evaluation of the upland weed control potentiality of green tea waste—Rice bran compost and its effect on spinach growth. *Am. J. Agric. Biol. Sci.* **2007**, *2*, 142–148. [[CrossRef](#)]
60. Mujtaba, G.; Hayat, R.; Hussain, Q.; Ahmed, M. Physio-chemical characterization of biochar, compost and co-composted biochar derived from green waste. *Sustainability* **2021**, *13*, 4628. [[CrossRef](#)]
61. Saini, A.; Aggarwal, N.K.; Sharma, A.; Yadav, A. Actinomycetes: A source of lignocellulolytic enzymes. *Enzym. Res.* **2015**, *2015*, 279381. [[CrossRef](#)] [[PubMed](#)]
62. Tortosa, G.; Castellano-Hinojosa, A.; Correa-Galeote, D.; Bedmar, E.J. Evolution of bacterial diversity during two-phase olive mill waste (“alperujo”) composting by 16S rRNA gene pyrosequencing. *Bioresour. Technol.* **2017**, *224*, 101–111. [[CrossRef](#)]
63. Martins, L.F.; Antunes, L.P.; Pascon, R.C.; De Oliveira, J.C.F.; Digiampietri, L.A.; Barbosa, D.; Peixoto, B.M.; Vallim, M.; Viana-Niero, C.; Ostroski, E.H.; et al. Metagenomic analysis of a tropical composting operation at the São Paulo zoo park reveals diversity of biomass degradation functions and organisms. *PLoS ONE* **2013**, *8*, e61928. [[CrossRef](#)]
64. Bosma, E.F.; Van De Weijer, A.H.P.; Van Der Vlist, L.; De Vos, W.M.; Van Der Oost, J.; Van Kranenburg, R. Establishment of markerless gene deletion tools in thermophilic *Bacillus smithii* and construction of multiple mutant strains. *Microb. Cell Factories* **2015**, *14*, 99. [[CrossRef](#)]
65. Yang, C.; Zhang, W.; Liu, R.; Li, Q.; Li, B.; Wang, S.; Song, C.; Qiao, C.; Mulchandani, A. Phylogenetic diversity and metabolic potential of activated sludge microbial communities in full-scale wastewater treatment plants. *Environ. Sci. Technol.* **2011**, *45*, 7408–7415. [[CrossRef](#)]
66. Pellegrini, M.; Spera, D.; Ercole, C.; Del Gallo, M. *Allium cepa* L. Inoculation with a consortium of plant growth-promoting bacteria: Effects on plants, soil, and the autochthonous microbial community. *Microorganisms* **2021**, *9*, 639. [[CrossRef](#)]
67. Tungmunnithum, D.; Thongboonyou, A.; Pholboon, A.; Yangsabai, A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines* **2018**, *5*, 93. [[CrossRef](#)]

68. Kumar, A.; Singh, S.; Gaurav, A.K.; Srivastava, S.; Verma, J.P. Plant growth-promoting bacteria: Biological tools for the mitigation of salinity stress in plants. *Front. Microbiol.* **2020**, *11*, 1216. [[CrossRef](#)]
69. Beckers, T.; Burkhardt, C.; Wieland, H.; Gimmnich, P.; Ciossek, T.; Maier, T.; Sanders, K. Distinct pharmacological properties of second generation HDAC inhibitors with the benzamide or hydroxamate head group. *Int. J. Cancer* **2007**, *121*, 1138–1148. [[CrossRef](#)] [[PubMed](#)]
70. Liu, H.; Guo, J.; Qu, J.; Lian, J.; Jefferson, W.; Yang, J.; Li, H. Catalyzing denitrification of *Paracoccus versutus* by immobilized 1,5-dichloroanthraquinone. *Biodegradation* **2011**, *23*, 399–405. [[CrossRef](#)]
71. Clark, L. DigitalCommons@University of Nebraska-Lincoln: A Review of Pathogens of Agricultural and Human Health Interest Found in Canada Geese. Available online: [https://digitalcommons.unl.edu/icwdm\\_usdanwrc/205/](https://digitalcommons.unl.edu/icwdm_usdanwrc/205/) (accessed on 21 June 2021).
72. Abdel-Monaim, M.F. Improvement of biocontrol of damping-off and root rot/wilt of faba bean by salicylic acid and hydrogen peroxide. *Mycobiology* **2013**, *41*, 47–55. [[CrossRef](#)] [[PubMed](#)]
73. Palaniyandi, S.A.; Yang, S.H.; Zhang, L.; Suh, J.-W. Effects of actinobacteria on plant disease suppression and growth promotion. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 9621–9636. [[CrossRef](#)]