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RESEARCH

Simulatenous evaluation of composting experiments and metagenome analyses to illuminate the effect of *Streptomyces* spp. on organic matter degradation

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Abstract

The effect of *Streptomyces* spp. on organic matter degradation was investigated in the present study. *Streptomyces* spp. isolated from compost systems were eliminated based on the results of cellulose, starch, xylan degradation tests, morphological inspection, and 16S rRNA analysis. The eliminated strains were re-given to compost systems to determine their effect on organic matter degradation and maturation. Sample analyses indicated that 15 days of composting had been adequate to maintain maturation. The amounts of strains added to the system were high enough to create a detectable change such as inhibition of other microbiota members. Results also indicated a variant change in organic matter degradation due to the added strain. The difference in organic matter degradation between strains depended partially on the segregation of secondary metabolites. On the other hand, strains also inhibited each other in the case of their binary and triple utilization in compost. Another explanation for variant activity was provided based on the enzymatic activity of the strains validated by metagenomic counts evaluation. Metagenome count numbers revealed the tendency of compost microbiota toward degradation products of cellulose. Findings obtained from composting experiments and metagenome analyses indicated the presence of a different degradation route based on xylan activity. Results also implied a decrease in competition between the dominant strain and microbiota members in the case of sequential xylan and cellulose degradation. Meticulous evaluation of results obtained from metagenome analysis also provided some insights on certain conditions regarding the progress of composting along with storage conditions of manure before use.

Keywords Compost · Cellulose · Metagenome · *Streptomyces* spp. · Xylan

Introduction

The wastes obtained as a result of domestic and agricultural activities have become a major issue due to the increase in world population, especially in the last century. The need to dispose of or transform waste materials into stable products resulted in the growing interest in a process called composting. This process contained the degradation of organic wastes via beneficial microorganisms and in the end, a stable, reusable product was obtained and utilized in agriculture (Lleo et al. 2013; Ouni et al. 2013).

Composting is a sequential process involving temperature intervals. Mesophilic (20–45 ℃) and thermophilic phases (45–65 ℃) both are conducted in the presence of beneficial microorganisms with optimum growths of 30–39 ℃ and 60 ℃ was generally accepted as the threshold of growth for thermophilic species. Biodegradable wastes are mostly removed in the thermophilic phase hence temperatures

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reaching at least 60 ℃ were expected during the process (Li et al. 2013; Lleo et al. 2013).

Compost quality is mostly governed by the structure of biodegradable products. Hence various types of wastes including paper, cellulose, and starch could be added to compost piles already containing agricultural and domestic wastes (Hermann et al. 2011; Biyada et al. 2022). Recent investigations on composting revealed higher efficiency compared to alternative methods (Hermann et al. 2011) and the sustainability of the process with minimal impact on the environment (Andersen et al. 2011; Saer et al. 2013; Jara-Smaniego et al. 2017).

Investigations on composting mostly included the effects of certain composting parameters along with constituents. However, recent studies involved the identification of microbial communities. These were identified to improve composting process towards a higher quality of the end product. A wide variety of microorganisms exists in the compost mixture. The dominant species depended heavily on the constituent of the compost mixture. A model sample obtained from a wastewater treatment facility in Finland was utilized as a microorganism source. Statistical analysis revealed the presence of over 2000 different species. On the species level, results were mainly affected by flora, region, and type of waste utilized for identification. On the other hand, the change in microbial diversity at the family level was found to be negligible based on the results (Partanen et al. 2010). This finding from the literature implied a negligible effect of composting parameters on diversity. Sugar beet pulp residue was given as another recent example and its effect on microbial diversity was investigated during the production of the genus *Agaricus brasilienses* (an edible mushroom) (Silva et al. 2009). Results validated the dominance of *the Bacillaceae* family followed by the Actinomycete group, mainly the *Streptomyces* genus (Silva et al. 2009).

The dominance of Actinobacteria in compost structure seemed to be a common situation based on a literature survey. The density of Actinobacteria could reach as high as 50% of the total microbial population at the end of composting (Steger et al. 2007). A similar result was previously provided by Wang et al. with composting of rice straw in nylon bags. Microorganism profile obtained via metagenome analysis showed that the Actinobacterium phylum had been predominant among bacteria (Wang et al. 2016). 16S rRNA analyzes of 41 isolates taken from 21 different compost samples used in mushroom production were performed and the genetic diversity of thermophilic Actinomycetes was examined in another study. Results validated the presence of five different genera belonging to the Actinomycete family as the major contributors to the thermophilic phase. Among these genera, *Streptomyces* and *Thermoactinomycete* genera were found to be higher in density compared to others (Song et al. 2001). Rice residue and unburned manure utilized as compost ingredients were randomly sampled during 0, 12, 42, and 112 days of composting. Firmicutes and Proteobacteria, which are members of the *Bacillus* genus, were determined as the most common phyla. However, results indicated the dominance of the Actinobacteria phylum in the thermophilic stage (Tian et al. 2013).

Inoculation of microorganisms inside the compost mixture was a relatively recent approach to enhance organic matter degradation during the process. Previously utilized microbial inoculums included Actinomycetes along with cellulolytic thermophilic actinomycetes. Cellulose and lignocellulose degradation, enzyme activities, and microbial community were previously evaluated as system responses. Results indicated that actinomycetes inoculation had simultaneously altered bacterial community and accelerated degradation of cellulose, hemicellulose, and lignin structures (Wei et al. 2019). Compost samples treated by a microbial column were stated to increase the efficiency of ocra (*Albemoschus esculentus*) and corn (*Zea mays*) when applied to soil (Asadu et al. 2018). Microbial inoculation was also reported to enhance cellulase activity, amount of humic substances, and degradation of cellulose (Zhao et al. 2017).

Based on the literature survey microbial inoculation would be effective both in compost quality and crop efficiency when utilized during composting. Actinobacteria was proven to be a dominant constituent of microbial diversity based on the outstanding performance of the phylum during composting. The decrease in composting time and/or increase in the amount of degraded organic matter could be achieved by the addition of proper *Streptomyces* spp. during composting. Completion of either of these goals was intended in the present study. Cellulosic and xylanolytic thermophilic Actinobacteria isolated from various compost samples were regiven to compost systems and their effects on composting performance and quality were determined by a series of analyses. Metagenome analyses were also conducted to evaluate the effects of microorganism addition on the microbiota.

Methods

Preparation of compost mixtures for use in thermophilic Actinobacteria spp. isolation

Despite the possibility of a decrease in microbial diversity, compost mixtures were utilized as sole isolation media to ensure the acquisition of thermophilic Actinobacteria spp. Hence two different compost mixtures were prepared solely for isolation. The first mixture consisted of manure, activated sludge, and wheat straw, while the second mixture was prepared by adding soil to the wheat straw and manure. Activated sludge and soil were solely utilized as microbiota

to facilitate isolation. Elemental analyses were conducted on each component before composting and the amounts to be used in the compost mixture were adjusted based on the C/N ratio of 30/1. The total weight of the compost was determined as 300 kg on a dry basis.

The components of the compost mixtures prepared for the isolation of microorganisms are given in Supplementary Table 1. The thermophilic phase was monitored with measurements of temperature, pH, and moisture values (Supplementary Table 2). Sampling was initiated as soon as the temperature value reached 45 °C. Samples were obtained with a temperature difference of less than 5 °C. These were then combined and evaluated as one sample. Sampling utilized for isolation was conducted from various layers (top, middle, and core of compost pile) and a total of 10–15 subsamples were collected. Samples obtained with a temperature difference of less than 5 °C were combined and evaluated as one sample for measurements. Analyses, pH and moisture measurements were conducted solely for monitoring the course of composting which was evaluated as the indicator of smooth microbial activity.

Isolation of thermophilic Actinobacteria spp.

Temperature monitoring was conducted on 2 systems prepared as duplicates. Combined samples were acquired in temperature intervals of 45–50, 50–55, and 60–65 °C. Samples were used in selective isolation studies with 4 different media and different selective agents. The media and antibiotics used were given in Supplementary Table 3. Isolation plates prepared according to the dilution plate technique were incubated at 45, 50, 55, and 60 °C for 7–10 days (Sembring 2000; Koçak 2019).

Cellulose/starch/xylan degradation tests for the selection of *Streptomyces* to be used in compost mixture

Cellulose degradation test

The isolates were inoculated on CMC agar (tryptone, yeast extract, NaCl, CMC, Agar) medium (pH 10.0 and containing 10% NaCl) and incubated at 45, 50, 55 ve 60 °C for 7–14 days. At the end of the incubation, 0.1% congo red solution was poured into the petri dish and the samples were stained for 15 min. Excess solution was washed with 0.1 N NaCl and petri dishes containing isolates were hold at temperature of inoculation for another 15 min. Strains showing yellow hydrolysis zone were considered to be active towards cellulose (glucanase) degradation (Hong et al. 2018; Hwang et al. 2018).

Starch degradation test

The starch degradation test mainly consisted of test strains' incubation at 45, 50, 55, and 60 °C for 5 days. Strains were incubated in Petri dishes containing starch (0.1% w/v) added glucose yeast extract agar (GYEA) (Cowan and Steel 1965). At the end of incubation, Lugol's iodine solution was poured into Petri dishes to form a thin layer on the medium. The presence of oligosaccharides and other simple sugars in the medium was determined by observing the open zone around the growth area which was evaluated as a positive indicator of starch degradation.

Xylan degradation test

The xylan degradation activities of the isolates were determined according to the procedure established by Voget et al. Specimens inoculated in streaks on "Oat Spelt" medium containing xylan were incubated overnight at 37 °C (Voget et al. 2006). At the end of the incubation, 0.1% Congo red solution was added to the medium, and staining was performed for 15 min. At the end of the staining period, 1 M NaCl solution was added to the medium to remove excess dye. Petri dishes were kept for another 15 min for observation of a yellow hydrolysis zone around the isolates, as an indicator of xylan degradation (Voget et al. 2006).

Application of morphological tests to selected isolates

ISP media (ISP 2–7 agar) and other media (Nutrient Agar, modified Bennett's agar, trypticase soy agar, Czapek's agar) were used for morphological examination of Actinobacteria spp.

DNA isolation, sequencing and phylogenetic analysis for 16S rRNA analysis

DNA of *Streptomyces*-like isolates was obtained using the DNA Isolation Kit. Amplification of the DNA region encoding the 16S rRNA gene was achieved using two universal primers (27f and 1525r; Lane 1991). Sequencing of the 16S rRNA gene region via five different oligonucleotide primers (Supplementary Table 4) was performed by Service Procurement (MacroGen Inc., The Netherlands). The 16S rRNA sequence datas were aligned in the MEGA X program and 16S rRNA nucleotide similarity with the most closely related organisms was determined using global alignment algorithms available on the EzTaxon Server (http://eztaxon-e.ezbiocloud.net; Kim et al. 2012). Neighbor-Joining (Saitou and Nei 1987) algorithm and Jukes-Cantor evolutionary distance matrix were used to plot phylogenetic dendrograms (Jukes and Cantor 1969).

Preparation of compost systems/determination of microorganism effect on composting

Systems were prepared based on strains' single, double and triple addition to compost mixtures along with a control system prepared without microorganism addition. A total of eight systems with two replicates were investigated in the study.

The primary aim in preparing composting systems was to determine the effect of strains when added to compost mixture and hence the compost preparation method, the analyses, and measurements of samples conducted during trials were configured accordingly. Windrow composting, also known as periodically turned static pile composting was applied in the course of experiments. Turning of compost mixtures should be evaluated as a separate parameter and its effect, in our opinion, should be investigated in a separate study. However, as stated, the primary aim was to detect the effect of microorganism addition, and hence turning was merely applied to serve acceleration of biomass decomposition through aeration. 300 kg in dry basis accounted for approximately 500 kg in wet basis forming piles that could reduce heat loss inside the mixture. Sampling during the trials was conducted from various layers (top, middle, and core of compost pile) and a total of 10–15 subsamples were combined for measurements and analyses. The turning interval was adjusted to 3 days for all compost systems. Turning and sampling procedures applied in the course of the study were compatible with EPA (40 CFR Part 503) requirements (Kumas et al. 2021).

Temperature measurements were conducted at 24 h intervals while pH and moisture values were monitored before aeration. Moisture contents for various systems dropped below the threshold (> 40%), however, the only intervention applied to systems was aeration to achieve a comparison between microorganism(s) (See Supplementary Table 5).

Strains, added to compost mixtures were coded as A, B, and C to provide easy follow-up. Hence compost systems were named accordingly. Strains were obtained via lyophilization with a total amount adjusted to 1 g. The number of colony units per milliliter (CFU/mL) was determined using plate count method. 0.1 g of lyophilized sample was serially diluted with 4900 µL nutrient broth. 200 µL from each dilution were inoculated on nutrient agar and plates were incubated at 30 ℃ for 96 h. The addition was performed with a concentration of 1 g/L prepared with ringer solution. Hence besides single utilization, the amount of strain added to systems for binary (AB, AC, BC) and triple (ABC) mixture was 0.5 and 0.33 g/L, respectively.

Investigation of the effect of strains on compost maturation

Mass reduction due to organic matter degradation was evaluated as a strong indicator of the microorganism effect during composting. Hence the change in organic matter % was the first set of measurements conducted during composting trials. Organic content was determined via a combination of subsamples periodically collected during composting. Samples were consecutively dried at 105 ℃ and ashed at 550 ℃ for 4 h (Kumas et al. 2021). The change in organic matter % was determined based on the difference between the values calculated at the 0th and 15th days of composting. The determination of germination index (GI %) was also adopted as a plant-based approach to evaluate the effect of degraded compost (Pampuro et al. 2017; Jagadabhi et al. 2019). GI % values were determined with 20 g samples diluted with 200 mL of de-ionized water for 5 h. The extract obtained after centrifugation at 10,000 rpm was utilized with cucumber (*Cucumis sativus*) and wheat (*Triticum*) seeds. The cucumber was selected for its sensitivity to NH_3. The wheat seeds utilized in the course of the study were known as "Ahmet" species, a local stock being protected and in danger of extinction. 5 mL of compost extract was added to Petri dishes containing seeds placed on sterilized "Whatman No 1" filter paper. Each petri dish contained 10 seeds with 3 replicates and technical repeatability was also ensured with separate runs conducted under identical conditions. A set of experiments was also conducted to determine the toxic effect of compost extract on seeds. Experiments performed under identical conditions included inoculation of seeds in compost extract for 2 h. This set of experiments was different from those conducted for the utilization of compost extract. In this seperate case, seeds inoculated with compost extract were added to Petri dishes. Filter paper in Petri dishes was wetted with 5 mL of de-ionized water. This set of experiments (both for wheat and cucumber) was called "inoculation" to ensure easy follow-up. GI values (Eq. 1) were calculated based on a comparison with de-ionized water-added seeds (Pampuro et al. 2017; Jagadabhi et al. 2019; Tong et al. 2019) according to:

$$GI(\%) = ((SG(\%) * RL(\%))/100 \quad (1)$$

where GI (%): germination index, SG (%): seed germination % compared to control, RL (%): mean root length compared to control.

A group of analyses was also conducted to evaluate the effect of microorganism addition on compost maturation. Samples obtained at the end of 15 days of composting were analyzed to determine Total Nitrogen, C/N ratio, and Ammonium/Nitrate ratios. T values were also calculated

as the ratio of initial C/N to C/N at the end of 15 days of composting. The change in C/N ratios and Ammonium/Nitrate ratios at the 3rd, 9th, and 15th days of composting were determined for systems prepared with single microorganism addition.

Antogonistic effect among *Streptomyces* used in composting

The antagonistic effect of Streptomyces used in composting was determined by the "Cross-streak" method to evaluate the compatibility of possible microbial consortiums (Dede et al. 2020). This test was added to the study based on the results obtained with compost samples prepared in the presence of binary and triple microorganisms.

Determination of microbial interaction in composts by metagenome analysis

Genomic DNA isolation of samples taken from compost was prepared by "Quick-DNA TM Fecal/Soil Microbe Miniprep Kit, Cat. No.: D6010". The amount and purity of the isolated DNA were determined fluorometrically by Qubit. The V3–V4 regions of the 16S rRNA gene to be used for species determination were amplified with 341F-805R primer sequences using SimpliAmp Thermal Cycler. PCR reaction mix and reaction conditions are given in Supplementary Tables 6 and 7.

Library preparation for 16S rRNA V3-V4 amplicon products Illumina's "Nextera XT DNA Library Prep Kit, Cat. No.: FC-131-1096" and indexing with "TG Nextera XT Index Kit v2 Set A (96 Indices, 384 Samples), Cat. No.: TG-131-2001". PCR purification processes were performed with "AMPure XP beads" from Beckman Coulter. Sequencing was done with Illumina's Miseq platform as paired-end (PE) 2×150 base reads. A minimum of $\geq 30{,}000$ readings were obtained per sample.

Raw data reads (FASTQ) were QC checked, trimmed (if necessary), and OTU graded with the Kraken Metagenomic system (Wood and Salzberg 2014). The statistical analysis of Shannon and Simpson indexes was performed by comparing compost systems (Systems A, B, and C) with control at the beginning (0th day) and on the 15th day. In other words, samples collected at the end of 15 days from systems A, B, and C were compared with samples obtained from the control system at the beginning (0th) and at the 15th days. The statistical analyzes were evaluated as mean ± standard deviation (SD). Tukey's multiple comparison test (one-way ANOVA) was conducted to evaluate alpha variations between experimental groups. The degree of significance was shown as $*p < 0.05$, $***p < 0.001$ and $****p < 0.0001$. Heatmaps was constructed by GraphPad Prism 8 (GraphPad Software, USA) software.

Results and discussion

Preparation of compost mixtures for use in thermophilic Actinobacteria spp. isolation

The component ratios in the compost mixtures were determined before mixing and their moisture contents were illustrated in Supplementary Table 8.

Monitoring of parameters was carried out for two systems prepared sequentially, with six repetitions at three different points close to the center in each system. Temperature, moisture, and pH values obtained in compost systems are given in Supplementary Table 9 for manure, straw, and activated sludge, and in Supplementary Table 10 for manure, straw, and activated sludge, respectively.

Isolation of thermophilic Actinobacteria spp.

The dilution plate method was used in selective isolation studies with four different media and different selective agents. In isolation studies, a total of 476 Actinomycetes and *Streptomyces*-like isolates were obtained. Some examples of the isolation plates obtained are given in Supplementary Fig. 1, and the list of all isolates was illustrated in Supplementary Table 11.

Cellulose/starch/xylan degradation tests for the selection of *Streptomyces* to be used in compost mixture

Cellulose, starch, and xylan degradation tests were applied to 130 isolates selected according to their microscopic and morphological characteristics. Strains with yellow hydrolysis zone in cellulase activity were evaluated as cellulase (glucanase) positive (Supplementary Fig. 2). The ability of selected isolates to degrade starch (0.1% w/v) was examined using GYEA (glucose yeast extract agar) supplemented with starch (0.1% w/v). Images of enzyme tests were given in Supplementary Fig. 3, and test results including all isolates were given in Supplementary Table 12.

Test results were adopted as an elimination process in the course of selecting *Streptomyces* spp. appropriate for composting. Consequently, 56 isolates were positive for starch degradation, 20 isolates could degrade cellulose and 14 isolates were determined to have both properties. Strains with a yellow hydrolysis zone around the colony were evaluated as xylanase positive (Voget et al. 2006) and only two *Streptomyces* isolates coded N4A13 and T4A05 gave positive results. The results obtained are shown in Supplementary Table 12 and some examples of xylanase test were given in Supplementary Fig. 4.

Application of morphological tests to selected isolates

The morphology results of 21 isolates selected according to enzyme tests were given in Supplementary Table 13. Among the isolates examined in terms of substrate, air mycelium structures, diffusible pigment, spore morphology, and colony morphology, 15 of them were selected for 16S rRNA analysis.

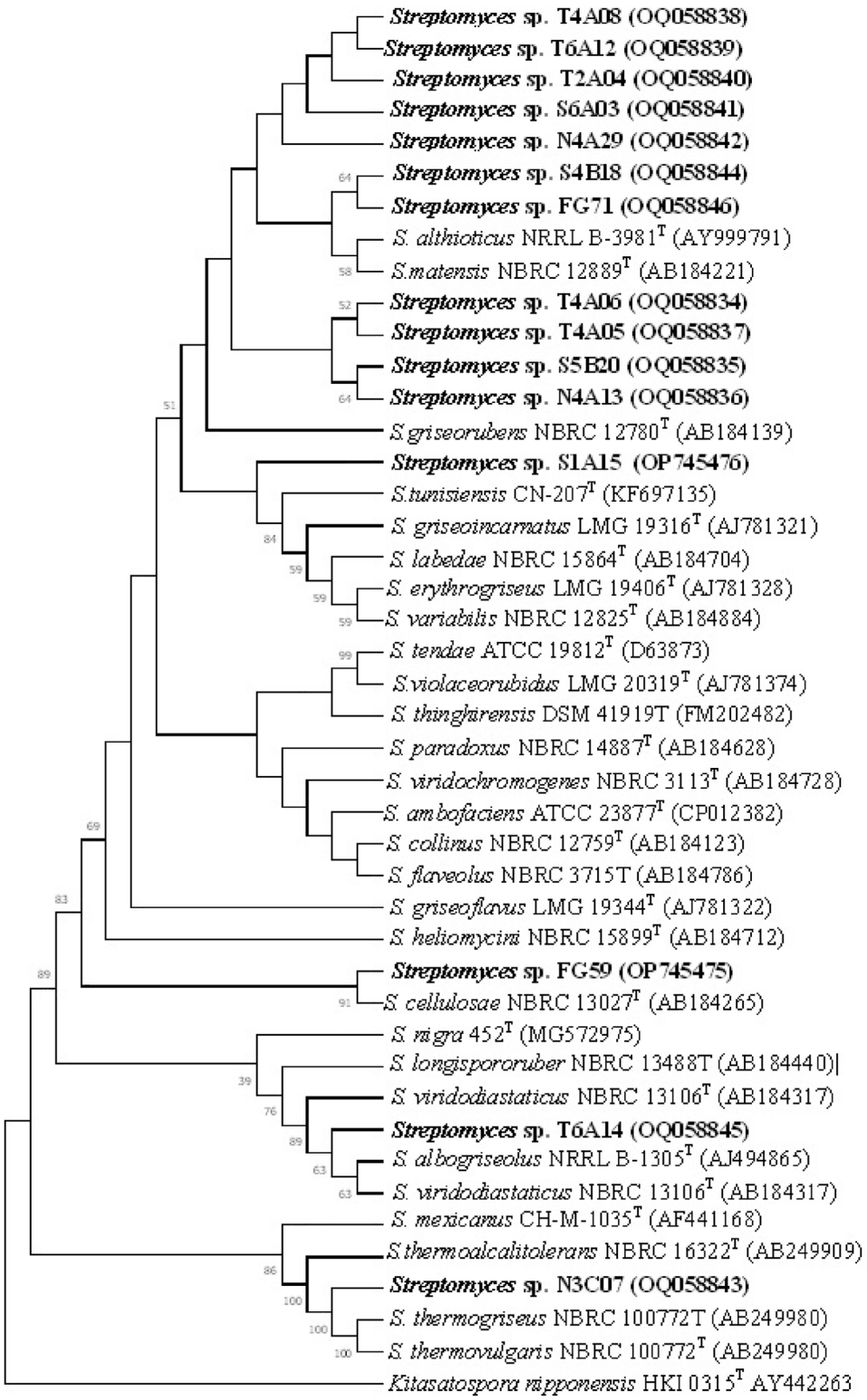

Fig. 1 Neighbor-joining (Saitou and Nei 1987) phylogenetic tree based on 16S rRNA base sequence analysis of test organisms and type species belonging to the genus *Streptomyces*. As an outgroup, *Kitasatospora nipponensis* (AY442263) is used. Strains' accession numbers were included in phylogenetic tree

DNA isolation, sequencing and phylogenetic analysis for 16S rRNA analysis

Phylogenetic dendrograms were constructed using the Neighbor-Joining algorithm and the Jukes-Cantor evolutionary distance matrix. FG71 and S4B18 sp. isolates were determined to have 99.93–100% similarity and 0–1/1433 nt difference with *Streptomyces matensis* (Fig. 1). N4A29, S6A03, T2A04, T4A08, T6A12, N4A13, S5B20, T4A05, and T4A06 isolates were determined to have 99.93–100% similarity, and 1–0/1447 nt difference with *S. griseorubens*. S1A15 isolate was also found to be closely related to *S. griseorubens* (98.76% similarity; 18/1447 nt difference). T6A14 isolate was determined to be related to *S. albogriseolus* type species with 99.93% similarity and 1/1449 nt difference. N3C07 sp. isolate had 100% similarity to *S. thermovulgaris* with a 0/1427 nt difference. FG59 isolate was identified as related to *S. griseoincarnatus* type strain (98.94% similarity and 15 nt difference) (Fig. 1).

N3C07 was the sole thermophilic species based on 16S rRNA analysis. Isolation was conducted at temperatures above the threshold of the thermophilic stage, however, it would be definitive to once more determine the temperature tolerance of strains and validate their convenience for use in composting. Hence a series of inoculation procedures were applied to selected strains in a temperature range of 4–60 ℃ to evaluate their compatibility during temperature changes of composting. Results given in Supplementary Table 14 indicated the majority of thermotolerant strains. Also taking into consideration that these isolates were obtained during the thermophilic stage of composting, the presence of thermophilic strains in a microbial consortium would have been necessary yet thermotolerant strains should reveal similar performance during the process. Results implied the scarcity of thermophilic strains even though isolation was conducted in the thermophilic stage, however, the results obtained in our study were not a unique situation based on recently obtained results in the study of Moreno et al. (2021). The development of 1380 bacteria and fungi in Moreno et al.'s study were examined between 20 and 60 ℃ and only 1% of the isolates were determined as thermophilic where as the ratio of thermotolerant species was 90%. In other words, the possibility of thermotolerant strains' isolation would be much higher compared to thermophilic strains which was the case in our study.

The results of enzyme tests, morphological tests, and 16S rRNA sequencing were evaluated together in the selection of microorganisms to be used in composting experiments. N4A13 and T4A05 coded isolates that were cellulase and xylan positive from enzyme tests and N3C07 coded isolates with 100% resemblance to thermophilic *S. thermovulgaris* sp. were chosen. Priority was given to the results of enzyme tests as in the case of N4A13 and T4A05 isolates closely related to the same type species with variant responses to enzyme tests, namely cellulose degradation. Another definitive feature of these strains was their performance in xylan degradation and taking into consideration that they had branched separately in the phylogenetic tree (Fig. 1), there had been a fair chance of them being separate species. The morphological features of these two organisms were evaluated as an additive conformation with the indication of different characteristics in different media (Supplementary Table 15).

Preparation of compost systems and monitoring of composting with measurements and analysis to evaluate the effect of microorganism(s) on composting

Evaluation of enzyme tests, morphological characterization, and 16S rRNA sequencing revealed three strains as potential candidates to be used in compost. Strains selected for compost trials were lypholifilized before addition. The colony numbers of lyophilize samples were determined by plate count method. A, B and C strains' colonies were enumerated. Results indicated $12–14 \times 10^8$ CFU/mL, $10–12 \times 10^8$ CFU/mL and $6–8 \times 10^8$ CFU/mL colonies for A, B and C strains, respectively. Lyophilized samples were added to the ringer solution and the total amount of strains was adjusted to 1 g/L. As previously stated, the amount of each strain was altered based on the number of utilized strain(s) which corresponded to 0.5 g for binary mixtures and 0.33 g for the triple mixture (See Supplementary Table 16).

The initial C/N ratio of compost mixtures prepared for microorganism isolation was 30/1, the same principle adopted for compost systems prepared for isolation was also applied for strain(s) inoculated compost systems. Hence total carbon amounts of manure, grass, and wheat straw utilized in composting experiments were determined as 32.6, 30.7, and 44.2% while nitrogen amounts were 1.1, 1.6, and 1.2%, respectively. These values corresponded to an initial C/N amount of 31. The amounts of manure, grass and wheat straw utilized in the compost mixture were 284, 1, and 15 kg, on dry basis.

Temperature values measured at 24 h intervals were evaluated as the first set of results to illuminate the effect of microorganism(s) on compost mixture. Results were given in Fig. 2 and Table 1, respectively.

An increase in temperature values was observed for all systems between 0 and 3 days. A gradual decrease in temperature values starting from the 9th day of composting could also be seen in Fig. 2. A high-temperature zone between 3 and 9 days was determined which seemed to be independent of microorganism addition. Results, at first sight, implied that microorganism addition was not effective on composting time. However, the evaluation of results as given in Table 1, indicated variances in thermophilic phase durations. Except for System C, the thermophilic phase was longer in microorganism-added systems compared to the

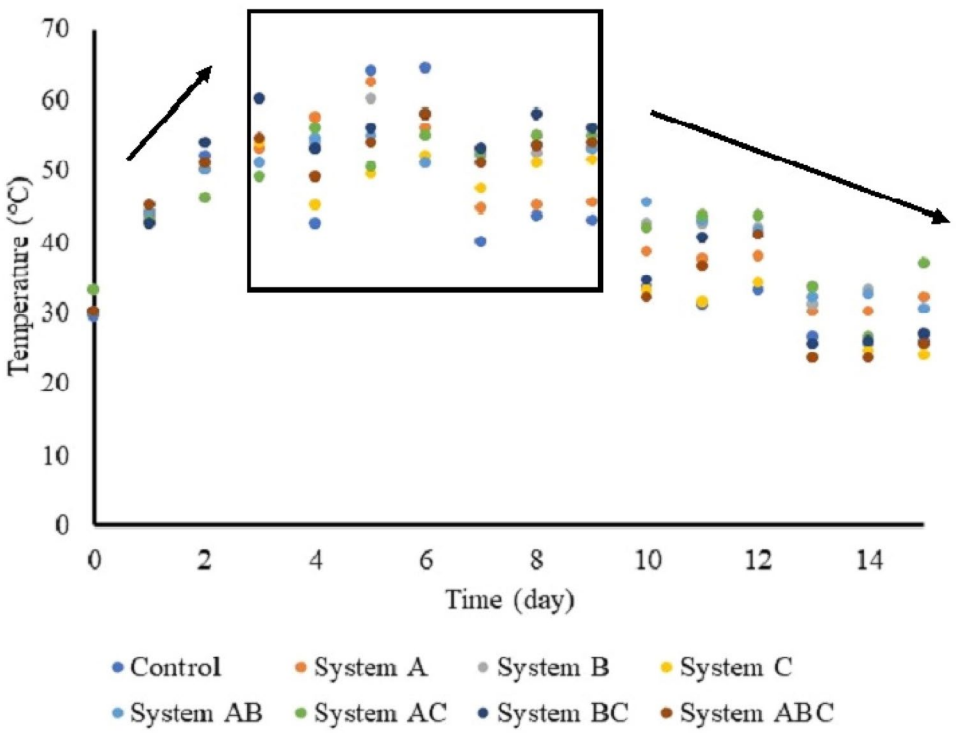

Fig. 2 Temperature variation of systems during 15 days of composting (Compost ingredients: manure, grass, wheat straw; C/N ratio: 30/1)

Table 1 Performance evaluation of compost systems during 15 days of composting

System	Compost initiation (°C)	Time to reach 55 °C (day)	> 55 °C duration (day)	Peak temp. (°C)	Time to reach peak temp. (day)	Thermophilic phase duration (day)*	Time to reach maturity (30 °C) (day)
K	30	3	4	65	6	10	12
A	30	3	4	63	5	13	13
B	30	4	3	60	5	13	15
C	30	3	1	55	3	10	14
AB	30	4	2	55	4	13	15
AC	33	4	6	56	4	13	15
BC	30	2	8	60	3	13	13
ABC	30	3	4	58	7	13	13

control. A comparison of peak values revealed an increase of ≥ 55 ℃ which was the threshold for compost sanitation (Inserra et al. 2006).

In our opinion, temperature measurements should be evaluated merely as an indicator of microbial activity. In the case of microorganism addition, the effect manifested itself as a general increase in the duration of the thermophilic phase, and results also implied a decrease in times to reach peak temperatures. Hence temperature measurements implied a noticeable effect of microorganism addition.

pH and moisture values were given in Supplementary Table 17. Results indicated a change of pH values between 9 and 9.5. These high values were expected considering the presence of manure with the highest amount in the compost mixture. Moisture contents were adjusted in threshold (40–50%) at the beginning of composting. Systems were compared for microbial activity which could be observed with changes in moisture contents. As seen from Supplementary Table 17, the gradual decrease of moisture for single strain-added systems was more evident compared to control, binary and triple systems. Nevertheless, results implied an elevated activity during composting which was attributed to microorganism addition. In other words, microorganism amounts utilized for composting were evaluated to be adequate to accelerate the composting process.

The organic matter of compost mixtures was determined via sampling every 3 days. Organic matter % amounts and % change of organic matter for 3-day intervals were given for

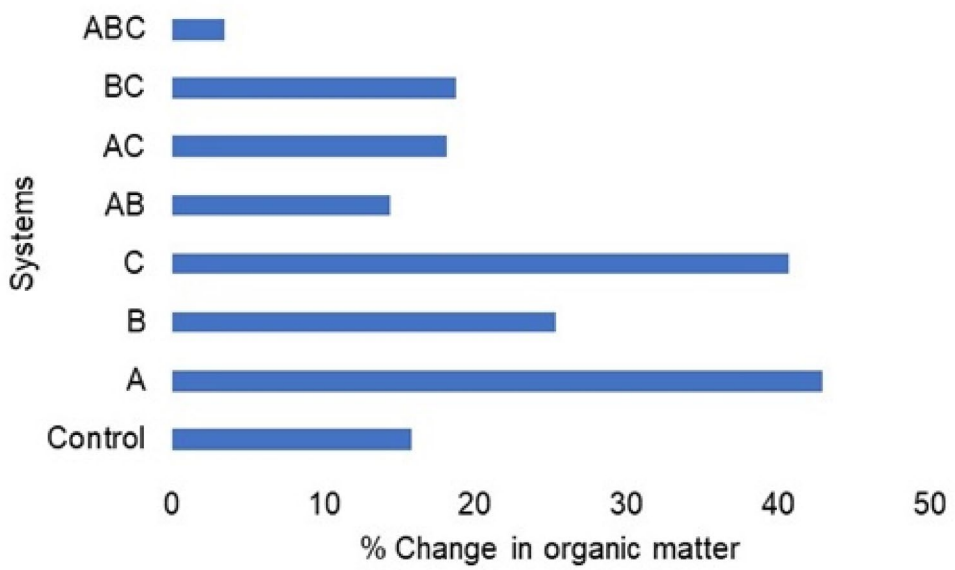

Fig. 3 Percentage change of organic matter in systems at the end of 15 days of composting

each system in Supplementary Fig. 5. Results were evaluated and illustrated in terms of organic matter change in Fig. 3. The change of organic matter was the highest in systems prepared by single microorganism inoculation. On the other hand, values close to control were obtained in the case of binary systems and the change of organic matter for system ABC was even lower than control. Results indicated a detectable effect of microorganisms which was prominent in the case of single utilization. Binary and triple utilization of strains, on the other hand, resulted in inhibition of activity which was thought to be due to antagonism between strains.

Antagonism between strains was an unexpected result considering the amounts utilized for the compost mixture. This result, however, also implied the effect of microorganisms on the compost mixture and revealed that the number of strains utilized for inoculation had been adequate to create an observable change in the course of degradation. Results obtained from Fig. 3, were almost in accordance with temperature measurements. The "almost" part was the effect of C strain which was one of the highest along with strain A. Temperature measurements indicated the lowest temperature values for system C and at first sight, the results obtained with temperature measurements and organic matter degradation were incompatible.

Organic matter degradation is often associated with temperature increase which, in our opinion, is a misconception. The increase in temperature values was due to a combined gradual microbial activity, in other words, a combined activity of inoculated strains and other members of the microbiota should be considered when evaluating temperature and organic matter degradation in accordance. Combined activity manifests itself in certain routes: organic matter degradation might lead to a temperature increase and microorganisms active for a certain temperature range would take part in composting creating a gradual increase in temperature. However, the amounts of strains added to the system were shown to be high enough to create a detectable change and this high amount could cause inhibition of other members that already existed in the microbiota. Inhibition could result in the restraining of other microorganisms' activity. There is also a possibility of an alteration in degradation products which could be the key factor in the inhibition of microbial activity. In other words, degradation of systems A and B could be beneficial for other microorganisms' activation which would lead to a higher increase of temperature in their presence. On the other hand, in the case of system C in compost mixtures, the organic matter could follow a different degradation route, the products of which could not be utilized by other microorganisms. In this case, considering a combined effect of inhibition and lack of useful ingredients for microbial growth, strain C, to a large extent, would be solely responsible for the observed activity.

Evaluation of results obtained from temperature, pH, and moisture measurements along with organic matter analyses revealed a certain effect of antagonism between the strains decreasing the activity. Another result, yet not certain but could be implied, was the possibility of varying degradation routes which could affect the activity of other microbiota members and hence affect the temperature profile. In other words, some properties of strains should also be accounted for to convert implication to a conclusion.

As previously stated strains B and C indicated identical resemblance to *Streptomyces griseorubens* with 99.93 and 100% resemblance, respectively. Their performances for organic matter degradation were also similar. The only difference between these two strains was detected from enzyme

tests. Strain B was found to degrade both cellulose and xylan, while strain C could only degrade xylan according to enzyme tests. Hence there was a strong possibility that these two strains had been different species.

The performance of organic matter degradation was evaluated based on the time of highest organic matter degradation. The time of highest organic matter degradation for strains B and C was 3 days. On the other hand, the time of highest organic matter degradation for strain A was 12 days. The difference in the highest degradation times could be explained by enzyme activities. Strain A could only degrade cellulose and based on the results the strains with xylan degradation capability could degrade organic matter faster than other strains.

Xylan degradation was considered a selection criterion for *Streptomyces* sp. since Actinobacteria had the highest number of members among other phyla that could degrade hemicellulose (Sriyapai et al. 2013). Xylanase was among the enzymes effective in degrading complex organic compounds to water-soluble compounds. As stated above, the time of highest organic matter degradation in the presence of strains B and C was 3 days and temperature values on the 3rd day of composting were determined as 51 and 54 ℃, for systems B and C, respectively. The highest xylanase activity was previously observed above 50 ℃ and pH values higher than 8 (Wang and Liang 2021) which was very similar to systems investigated in the course of the present study. In other words, suitable conditions for xylanase activity might be created during composting. A literature survey could bring an explanation for the highest activity of B and C in relatively shorter periods. However, a different perspective is required to explain the higher activity of strain C compared to B. Until now, the number of strains added to systems was concluded to single-handedly alter the course of organic matter degradation. In fact, the main effect presented via strains was mainly due to the enzymes secreted during composting. As previously stated, hemicellulose degradation resulted in the production of water-soluble compounds. These could be utilized as a food source by other members of the microbiota. Organic acids produced during the degradation of organic matter had an increasing effect on the degradation of xylan and cellulose, however, this increasing effect was higher in the case of xylan degradation. In other words, it would be logical to presume higher and more prominent activity of xylanase compared to cellulase in the early stages of composting (Pathak 2017). In a study where ground wheat straw was utilized as a growth medium for *Streptomyces* sp. results indicated higher utilization of hemicellulose (40%) compared to cellulose (5%) of wheat straw during *Streptomyces* sp. growth (Godden et al. 1989). Compost mixtures with very high amounts of strain that could solely control organic matter degradation would serve as growth media for *Streptomyces* sp. development. Hence mainly xylanase activity and negligible cellulose activity should be expected in the early stages of composting. Comparison of B and C for 15 days revealed higher amounts of degradation in the presence of the C strain. Based on the literature survey, we believed that hemicellulose degradation had mostly been favored during composting, higher activity in the presence of strain C could be explained by higher xylanase activity in its presence. However, xylanase activity could be inhibited due to temperature. Isolation of Strain C was conducted at 45 ℃ and strain B was isolated at 50 ℃. The highest composting temperatures obtained for both systems were 10 ℃ higher than the temperatures of isolation. Considering the limited tolerance of these two strains to high temperatures, it could be implied that both strains had certain control over the course of temperature. In other words, the highest temperatures reached during composting of systems B and C were thought to be the threshold of their growth. Under the circumstances where a 10 ℃ difference in temperature was observed for two systems, the variation in activity should be due to temperature, the most important inhibitor of xylanase activity. A decrease of 18% in xylanase activity was previously found at 55 ℃, and this decrease reached 55% at 60 ℃ (Sriyapai et al. 2013; Das et al. 2017). Consequently, higher xylanase activity should be expected in the presence of C based on the temperature differences between the two systems.

The highest organic matter degradation was found in the presence of the "A" strain. This strain was found to have a 100% resemblance with *Streptomyces thermovulgaris*. This species was previously stated to take part in organic matter degradation and this literature finding could partly explain its highest activity. The main difference between system A with systems B and C was the time to reach the highest degradation of organic matter which was the 12th day for system A. The highest temperature reached in the compost mixture was 63 ℃, which was close to the isolation temperature (60 ℃) of the strain. In other words, the conditions of the system were relatively favorable for strain A to thrive. The cellulose in wheat degrades slowly compared to hemicellulose. However, this slow degradation would serve a sustainable food supply which enabled slow, yet constant growth of microbiota.

Slow degradation of organic matter was an expected behavior in the presence of a strain with no xylanase activity. Hemicellulose and lignin resided as a protective amorphous cloth around the cellulosic structure and without xylanase activity, the interaction of cellulose secreted by the strain would be delayed. In the case of B and C strains, xylanase activity would serve as an enhancer for cellulose degradation which might be effective in accelerated activity in their presence (Ma et al. 2020).

In theory, combined utilization of a strain with cellulase activity and strain with xylanase activity should have a

higher or at least comparable effect in terms of organic matter degradation. However, that was not the case for the systems prepared with binary inoculation and organic degradation in their presence remained close to control. As an example, in system AC where a strain with high effect at low temperatures and a strain effective at higher temperatures were combined, the amount of organic matter degradation should be close to those obtained with inoculation of a single strain. Organic matter degradation was even lower than the control for system ABC. The number of microorganisms was high enough to create a change during composting, however, results obtained for binary and triple systems indicated that these strains had dominated the entire flora and their competition inside the compost mixture resulted in a deceleration of activity.

Antagonism of strains against each other was determined via the "cross-streak" method and results were illustrated in Supplementary Fig. 6. Results indicated inhibition of C in the presence of A, strains A and C could not thrive in the presence of B. When strain C was present in the media, the development of A and B was also not possible.

Table 2 Physicochemical properties of systems at the end of 15 day composting

System	Organic matter (%)	C/N	TN (%)*	Ammonium/ Nitrate	T value**
Kontrol	25.3	9.21	1.6	1.67	0.40
A	18.7	6.81	1.6	1.67	0.29
B	30.5	12.7	1.4	1.67	0.55
C	24.4	8.88	1.6	2.00	0.38
AB	27.62	11.5	1.4	1.33	0.50
AC	28.3	12.7	1.3	1.33	0.55
BC	27.3	9.91	1.6	1.50	0.43
ABC	32.8	12.7	1.5	1.25	0.55

*TN: Total Nitrogen (%), **Calculated via taking ratio of intial/final (15th day) C/N values

Consequently, these strains were shown to have difficulties in co-existing in the same media.

Investigation of the effect of strains on compost maturation

The physicochemical properties of compost mixtures determined on the 15th day of composting were illustrated in Table 2. C/N ratios varied between 9 and 13, as seen in Table. These values when compared to the literature were found to be more than adequate for maturity evaluation (Jiang et al. 2015; Cesaro et al. 2019). C/N values below 12 were previously stated as the threshold for negative impact on the microbiota. In other words, they could be added to the soil without further treatment. *T* values were also below the predetermined threshold (0.75) which was also evaluated as an indicator of maturity (Itävaara et al. 1997).

On the other hand, total nitrogen values varied between 1 and 2, and when evaluated with C/N ratios it was determined that nitrogen, available for plant use, would have been 10% of its original content. Consequently, results indicated the necessity of nitrogen addition along with compost application (Sullivan et al. 2018).

Ammonium/Nitrate ratios were also evaluated as an indicator of compost maturity. However, threshold values varied between studies, and values varying between 0.5 and 3 were accepted as a positive indicator of maturity (Teshome and Amza 2017). A specific value of 2.89 was previously obtained in the study of Martinez et al. which was accepted as adequate to conclude the compost as "mature" (Martinez et al. 2016). Based on these values Ammonium/Nitrate ratios varying between 1.25 and 2.00 in the present study were evaluated as a positive indicator of compost maturity.

C/N ratios and ammonium/nitrate ratios were also determined on the 3rd, 9th, and 15th days of composting for control, A, B, and C systems, and results were illustrated in Fig. 4. Results indicated a lower value of C/N ratio in the control system compared to others. Ammonium/Nitrate

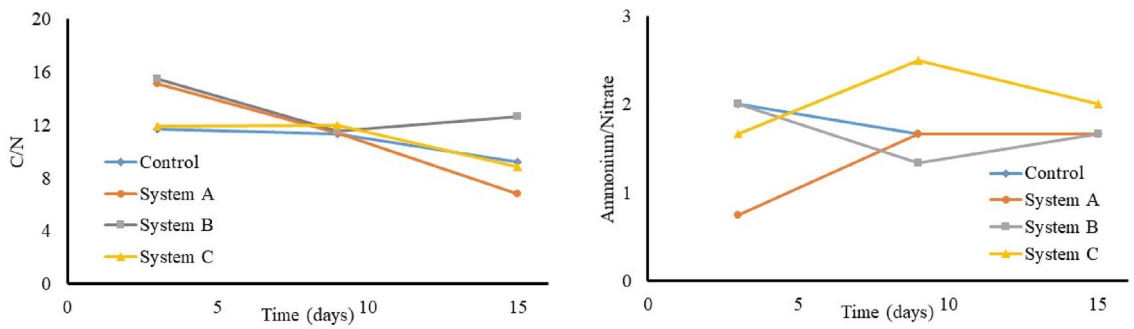

Fig. 4 The change of C/N and ammonium/nitrate ratios for single systems compared with control (Compost ingredients: manure, grass, wheat straw; C/N ratio: 30/1)

ratios of control were also lower compared to systems A, B, and C. These findings were in accordance with the change in organic matter. An ordered decrease of C/N ratios could be observed for control and system A which indicated steady, ongoing carbon mineralization. A negligible increase of C/N ratio between 3 and 9 days was detected for System C, however, the decrease between 9 and 15th days indicated a continuation of organic matter degradation within this system. In the case of System B, the increase of C/N ratios between 9 and 15th days was also evaluated as negligible. These increases in C/N ratios were previously explained with nitrogen reduction or the increase of total solids over time (Cesaro et al. 2019).

An increase of ammonium/nitrate ratios between 3 and 9th days was detected for systems A and C. Ammonium/nitrate ratio between 9 and 15th days were stable for System A, while a decrease was observed for System C. 3–9th days corresponded to an interval in which highest temperatures were observed during composting. Ammonia formation, a result of this mass microbial activity, was responsible for the observed increase in ratios. Temperature values decreased below the threshold of nitrification after the 9th day for systems A and C which implied acceleration of nitrification between 9 and 15th days.

A reverse trend was observed for systems B and Control. The decrease of the ratio between 3 and 9th days was thought to be due to the decrease of organic matter degradation. As stated, the highest change in organic content was observed on 3rd day for systems B and C, however, a closer look at temperature values indicated the formation of suitable conditions for xylanase activity in system C. Temperature values of System B, on the other hand, reached > 50 ℃ which decreased xylanase activity. Besides considering the temperature effect on ammonia volatilization interpretation of results implied a higher loss of ammonia than produced during composting. An increase of ammonia/nitrate ratios could finally be observed between 9 and 15th days for system B. This increase was thought to be due to temperature. In the case of system B temperature values below 40 ℃ could only be reached after the 12th day (Jiang et al. 2015). This late nitrification along with declined volatilization of ammonia was the key factor for the observed increase.

Germination Index values were obtained for cucumber (Cucumis sativus) and wheat (Triticum) seeds to determine the effect of composting on phytotoxicity. Experiments were performed with 2 runs with 3 replicates in each run to ensure technical and biological repeatability. Additional experiments with seeds inoculated in compost extracts were also conducted to further clarify the effect of compost extracts on phytotoxicity. Analyses were primarily evaluated to validate the harmlessness of microbiota that remained after 15 days of composting. Results were illustrated in Table 3. The fine performance of System C for both plants could be seen in the table. The germination index obtained for control, C, and ABC systems were shown to be higher than 80%.

Evaluation of the results obtained from C/N, Ammonium/Nitrate ratios along with calculated *T* values indicated the achievement of maturity in the presence of strains. Hence, a potential toxic effect determined during the evaluation of Germination Index values was attributed to the presence of ammonia. GI values obtained with System A, B, and BC were slightly below the threshold (Table 3). As previously stated, the root lengths of plants were also involved in the calculation of GI values. Hence root lengths for all experiments could be determined statistically (See Supplementary Figs. 7 and 8). Results indicated a slight decrease in the root length of the cucumber obtained after the addition/inoculation of System B's extract. Besides System B, root lengths obtained for systems generally remained unchanged implying that compost extracts were not harmful to the plants.

Although slightly below the threshold, the negative effect of Systems A and B implied a negative response of cucumber to the extracts obtained after composting in the presence of A and B strains. Before detailing the reasons for this negative effect, the response of wheat to the extracts was presented for comparison. Based on the results, the response of wheat to compost extracts was determined to be entirely

Table 3 Germination index % values obtained with wheat and cucumber

Sistem	Wheat (Compost)		Wheat (Inoculation)		Cucumber (compost)		Cucumber (Inoculation)	
	1st meas	2. meas	1st meas	2. meas	1st meas	2. meas	1st meas	2. meas
Control	84	80	104	101	108	115	83	95
A	98	93	105	90	75	84	72	76
B	102	80	125	79	68	72	82	83
C	89	87	87	115	101	103	85	82
AB	83	72	93	85	89	98	91	112
AC	129	67	148	94	81	89	80	88
BC	68	69	69	75	70	89	89	104
ABC	80	86	87	96	101	90	110	98

different from those obtained for cucumber. First of all, compost extracts obtained from binary systems were harmful to wheat as shown in Table 3. Besides, compost extracts obtained from single systems (Systems A, B, and C) resulted in high GI% values for wheat. The harmful effect of extracts obtained from binary systems could also be validated with a statistical decrease in root lengths in the case of wheat (See Supplementary Fig. 8).

The different responses of wheat and cucumber to compost extracts were thought to be due to the performances of strains during composting. Organic matter degradation for systems prepared with inoculation of single strains was as high as 40%. In the case of binary systems, these values remained between 15 and 20%. In other words, extract obtained from systems with almost complete organic matter degradation was harmful to cucumber. In our opinion, this sensitivity of cucumber emanated from the presence of ammonia rather than organic matter degradation (Roosta and Schjoerring 2007, 2008). High pH values indicated incomplete volatilization of ammonia during composting. Temperature values for systems A and B remained higher than 35 ℃, for 13 days. This relatively high temperature could inhibit nitrate formation from ammonia which in our opinion, resulted in high ammonia levels. In other words, volatilization of ammonia and/or formation of nitrate from ammonia was low in compost systems prepared with inoculation of single strains. In the case of binary systems lower ammonia formation should be expected related to lower organic matter degradation. Hence lower ammonia levels resulted in higher GI% values for cucumber.

The different responses of wheat to compost extracts also indicated an entirely different response of the plant to ammonia. Wheat-favored ammonia-based fertilizers enable the controlled release of nitrogen. A previous comparison of the effects of nitrate and ammonia-based fertilizers on wheat yield resulted in higher values in the presence of ammonia-based fertilizers (Çakır Ongören 2013). Hence compost extracts obtained from systems with complete organic matter degradation and higher ammonia levels could provide suitable conditions for wheat development. On the other hand, results obtained with binary systems implied the weakness of the plant to compounds obtained as a result of incomplete organic matter degradation. This implication should be validated with a separate study; however, the accumulation of soluble salts and organic acids was previously determined in the case of incomplete composting (Araujo and Monteiro 2005; Blewett et al. 2005). A literature survey also revealed the toxic effect of phenolic acids on wheat (Ozores-Hampton 1998). Based on these findings, relatively similar conditions leading to organic acid accumulation could exist especially in binary systems.

The overall performance of the C strain in compost was thought to be extraordinary compared to strains A and B. Compost system prepared with the addition of the C strain could barely reach the threshold temperature (55 ℃) of sanitation. Nevertheless, organic matter degradation in the presence of this strain was almost 3 times higher than control. System C was also the only system with germination index values higher than 80% for both wheat and cucumber. Despite high organic matter degradation, high GI % values for cucumber could be obtained with System C. This result indicated nitrate formation which was logical considering the lower temperature profile of this system compared to others. The performance of strain C was the most important finding obtained in the course of the study. Results indicated that reaching high-temperature values was not an obligation to ensure high organic matter degradation. In fact, temperature enough to meet sanitation requirements would be adequate for efficient composting as in the case of system C.

Antogonistic effect among *Streptomyces* used in composting

The results revealed that high degradation rates were achieved in the single use of microorganisms and that the microorganism had a visible effect on organic matter removal. Theoretically, it was thought that higher or at least comparable degradation rates with single microorganisms would be achieved with the combined use of microorganisms. For example, microorganisms added to the AC system functioned at both low and high temperatures, degrading cellulose as well as xylan. Therefore, the first impression was enhanced effect in the case of combined utilization. However, when the organic matter degradation rates were examined, values close to control were obtained in binary systems, while degradation rates only below 5% could be achieved in triple systems. These results were due to an antagonistic effect between strains. Antagonistic effect results were previously stated to illuminate the behavior of strains utilized in compost mixture (Supplementary Fig. 6). The test procedure was further developed for selected strains to determine their behavior toward both each other and towards other members of the *Streptomyces* genus. Results indicated that both A and C had inhibited the growth of B, and in a system composed of A and C strains, the effect of the strains on composting would probably be less pronounced due to their inhibiting effect on each other. Based on the results, the poorest performance should also be expected in the case of triple utilization.

The results of antagonistic effect tests could also be beneficial to envisage the activities of strains in the case of single utilization. Strain A and C inhibited close to 80% of other *Streptomyces* genus members while strain B showed a more compatible profile with only a 30% inhibition rate. These results implied that A and C, in the case of single utilization would dominate the flora. Hence antagonistic effect test was

evaluated as the final step in the validation of high activity observed with these strains (Supplementary Table 18).

Determination of microbial interaction in composts by metagenome analysis

The effect of strains on microbial microbiota could be envisaged via antagonistic effects. However, this was a limited approach between identical species, and a better means to evaluate the effect of strains on the microbiota should be conducted to fully understand their impact on composting. Hence metagenome analysis as a practical and more holistic approach was adopted in the course of the study. Organic matter degradation was higher in single strain-added systems, consequently, more emphasis on single systems compared to binary and triple systems was given in the evaluation. Metagenome counts in species level were given in Supplementary Table 19.

Statistical evaluation of diversity on the species level could best be performed via Shannon and Simpson indexes. Results illustrated in Table 4 indicated a decrease in both values at the end of 15 days. The decrease of both index values in the presence of strains was relatively prominent compared to the control at the end of the 15th day. This result implied the dominance of strains in compost mixtures. Shannon index values were higher than 3.5 regardless of strain addition which indicated high microbial diversity of compost mixtures (Feng et al. 2017).

The results obtained from statistical analyses were illustrated in Table 4. Statistically significant Shannon index values validated the increase of species richness due to a series of reactions resulting in the degradation of organic matter. Non-significant results obtained with Simpson index values implied an uneven distribution of species in the microbiota (Thukral 2017; Wagner et al 2018). Statistical analysis, indicated a gradual change of organic structure affecting the richness and distribution of species. Results obtained so far revealed the necessity of further evaluation of findings which was achieved by heatmap analyses Heatmaps prepared according to the counts of the highest 10 families, genera, and strain members were illustrated in Figs. 5, 6, 7. A drastic decrease in count numbers occurred after 15 days of composting regardless of strain addition to compost systems. This result was thought to be due to temperature increase creating suitable conditions for sanitation. However, a closer inspection of compost systems revealed that count numbers in the

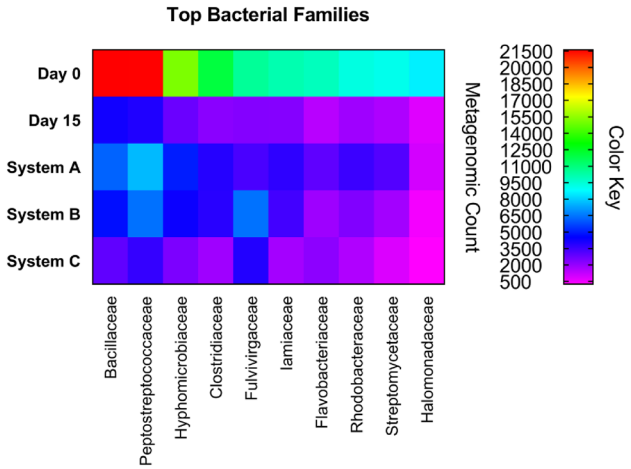

Fig. 5 Percentage distribution graph of the 10 highest bacterial families of all compost systems

Table 4 Dunnett's multiple comparison tests for statistical evaluation of Shannon indexes*

Shannon index			
Control 0th day vs			
Systems	Mean diff	Adjusted P value	Significance
A	0.1755	0.0016	**
B	0.4685	< 0.0001	****
C	0.5230	< 0.0001	****
Control 15th day	0.2670	0.0002	***
Control 15th day vs			
Systems	Mean diff	Adjusted P value	Significance
A	− 0.09150	0.0270	*
B	0.2015	0.0009	***
C	0.2560	0.0003	***
Control 0th day	− 0.2670	0.0002	***

*Simpson index values were found to be insignifcant

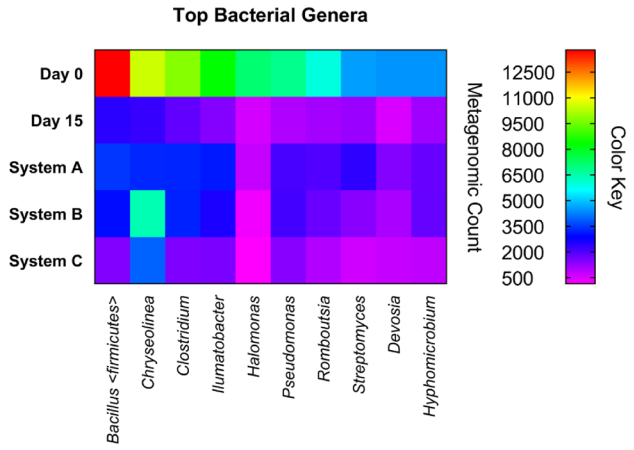

Fig. 6 Percentage distribution graph of the 10 highest bacterial genera of all compost systems

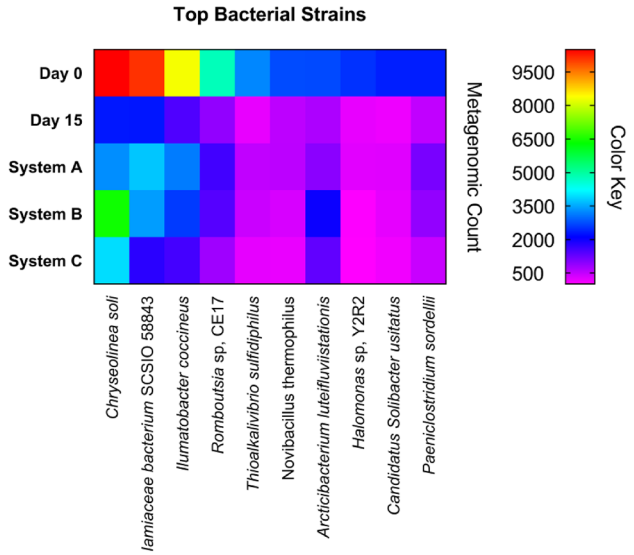

Fig. 7 Percentage distribution graph of the 10 highest bacterial species of all compost systems

presence of A and B strains had both been higher than on the 15th day of composting. This was among the highlights of the study as count numbers implied that degradation products of either A and B be utilized by the members of compost microbiota. However, a vice versa result, also a highlight, was obtained in the case of the C strain. Count numbers of System C, with few exceptions, had the lowest numbers among Systems A, B, Control (15th day), and C. This vice-versa result implied a strong inhibiting effect of strain C along with the presence of an entirely different degradation pathway. The presence of a variant degradation route should be expected considering that strain C had only xylan degradation ability.

A meticulous evaluation of heatmap analyses could provide some insights into possible situations that might have occurred in the presence of strains. Heatmap data were evaluated based on the majority in the presence of strains. Metagenomic counts revealed the highest numbers of *Micromonosporaceae*, *Flavobacteriaceae*, *Thermomonosporaceae*, *Streptomycetaceae*, *Microbulbiferaceae*, and *Bacillaceae* families in the presence of A strain. These families also had the highest count numbers among all strain and the difference in count numbers were mainly attributed to inhibiting the potential of strains. Count numbers of the family *Bacillaceae* provided a fine example of the inhibition potential of strains along with *Streptomycetaceae* which included the genus *Streptomycetes*. Count numbers of families *Bacillaceae* and *Streptomycetaceae* in the presence of strain A were at least 1.5-fold higher than the closest strain. The possibility of a certain inhibiting effect has best manifested itself via *Bacillaceae* count numbers. These family members were well known for their endurance at temperature, pH, and salt concentration extremes. The noticeable difference in count numbers was presented as a comparison to the inhibition potential of strains (Sangannavar et al. 2021). *Microbulbiferaceae* was another family with threefold higher count numbers in the presence of strain A. These family members could thrive in high-salt environments and are given as another example for the validation of milder conditions in the presence of Strain A.

Thermomonosporaceae majority was another important, yet expected result in the presence of strain A with the longest duration of high-temperature conditions during composting. As stated, compost system A also had one of the longest duration times at temperatures higher than 55 ℃ (Table 1) (Trujillo and Goodfellow 2015). A special emphasis on higher count numbers of *Flavobacteriaceae* should also be given since this family was determined in the presence of all strains with relatively closer numbers. Family members included physiologically diverse species all of which were strictly aerobic (Waskiewicz and Irzykowska 2014). Hence high count numbers of this family were evaluated as the presence of sustainable aerobic conditions during the composting process.

A general evaluation of metagenome counts revealed an undisputed majority of families in the presence of strain A. However, some families indicated higher count numbers in the presence of strain B. One example was the presence of the *Fulvivirgaceae* family. The metagenome count of family members was found to be at least two-fold higher in the presence of strain B. This family was also among the families with the highest count numbers. *Fulvivargaceae* was known as a member of the phylum Bacteriodata, the members of which could degrade complex sugars such as cellulose, starch, and xylan (Jehlicka et al. 2013). High count numbers of *Fulvivargaceae* should also be expected in the presence of

the C strain provided that the activity was identified solely by xylan degradation. However, that was not the case and as stated, the count numbers in the presence of the C strain had almost always been lower than A and B.

The inhibition potential of strains, based on findings, was certain. However, the factors creating this inhibition effect were not clear. As stated, all the strains utilized in composting were members of the *Streptomyces* genus recently identified as a "biofactory of secondary metabolites" in the work of Alam et al. (2022). Release of secondary metabolite inhibited the development of other *Streptomyces* spp. which was shown to be more pronounced in the presence of Strain C (Supplementary Fig. 6, Supplementary Table 18). Hence a variant release of metabolite depending on the strain might be an explanation for these variant count numbers. Another explanation might be the enzymatic activity of the strains and since the strains were added in high amounts, high enough to directly alter compost microbiota, enzymatic activity might also have a role in variant count numbers. In the case of strain B, combined cellulase and xylanase activities would lead to the sequential degradation of hemicellulose and cellulose. Degradation of hemicellulose via xylanase would also facilitate microorganisms' transport to cellulose. Hence degradation products in strain B's presence would be sufficient for family members utilizing complex carbon sources. In the case of C, degradation entirely depended on xylan, the products of which were not preferred based on count numbers. Strain A, on the other hand, degrades cellulose, and based on count numbers degradation products of cellulose were favored by the higher number of families (Fig. 5).

Consequently, the degradation of cellulose and xylan gave rise to family members having alternative preferences for degradation products, yet degradation products of cellulose were more favored by family members based on the results. This was an expected result considering the sampling environment which, in high proportions, consisted of manure.

Metagenome counts of genera indicated a trend similar to family counts, as expected. On the other hand, careful inspection of certain examples could provide insight into the conditions during composting. As an example, the *Magnetospirillum* genus had the highest density in the presence of strain B. The members of the genus were mainly characterized by their endurance in low oxygen even anaerobic concentrations (Schaechter 2009). The presence of *Magnetospirillum*, a member of gut microbiota, in the compost sample, could not be a coincidence. Considering certain functions of gut bacteria including xylan hydrolysis, high numbers obtained in the presence of strain B implied insufficient oxygen transfer to certain parts of the compost mixture which provided a basis for the development of anaerobic microorganisms also utilizing xylan as a carbon source (Yang et al. 2020). The highest *Magnetospirillum* count in the presence of B strain depended on the combined effect stated above. It was inferred from the results that aeration of compost piles should have been conducted more often to eliminate the occurrence of anaerobic conditions. In this context, high count numbers of the *Methylomicrobium* genus should also not come as a surprise considering genus members' methane utilization as an energy source (Berlanga 2010).

Another insight into enzymatic activity was obtained based on count numbers of the *Chryseolinea* genus. The metagenomic count obtained in the presence of strain B was also the highest number among other genera. This was an interesting result, yet a detailed investigation of the literature supplied a logical explanation for the highest count number obtained in the presence of strain B. *Chryseolinea* genus was known as a member of the Bacteroides phylum. A recently proposed species *Chryseolinea* was found to utilize xylan as a carbon source while species could not utilize CM-cellulose (Kim et al. 2013). Other members of the Bacteroides phylum such as *Bacteroides xylanisolvens*, as the name suggests, can dissolve xylan (Fijan et al. 2021). Hence results indicated the development of genus members when a decrease in rivalry conditions existed. This decrease was provided by the cellulose activity of strain B which enhanced the development of genus members due to the utilization of less preferred xylan as medium (Fig. 6).

Evaluation of systems at the species level provided more detailed conclusions on possible enzymatic interactions of strains affecting compost microbiota. As expected, the species with the highest count number was *Chryseolinea soli* obtained in the presence of the B strain. *Chryseolinea soli* was a recently investigated species indicating negative results for CM-cellulose hydrolysis. Results also indicated acid production in the presence of D-xylose (Lee et al. 2019). Hence it would be logical to assume the degradation of xylose residues, namely xylan. Enzymatic activity was effective in terms of increasing the diversity of degradation products. Cellulose degradation enabled a sufficient supply of media for other members' development whereas an extra source emanated from xylan degradation would enhance the thrill of microbiota members specifically/mainly utilizing this polysaccharide.

An elaborate evaluation of count numbers with composting experiments served as validation criteria for the stated results. *Steroidobacter denitrificans* as an example once again reached its highest numbers in the presence of the B strain. The distinguishing property of this species was its ability to utilize steroidal hormones, the possible presence of which was another matter of debate. The presence of steroidal hormones was a high possibility as hormones' degradation was due to nitrate reduction (Fahrbach et al. 2008) which was previously stated as a literature-based explanation for the increase of C/N ratio in system B (Fig. 4).

The presence of *Cellvibrio* sp. PSBB006 was emphasized as one of the most interesting results obtained via metagenome analyses. This species has been obtained in close count numbers regardless of inoculated strains during composting. A literature survey revealed the presence of species' genes related to xenobiotic degradation. In other words, the species would survive through rivalry with any strain. Xenobiotic degradation was evaluated as the preview of serious environmental pollution probably in the site where composts were collected (Breton-Deval et al. 2020) (Fig. 7). Metagenome analysis could also provide some clues on storage conditions of manure before composting. A unique bacterial profile among all systems was determined with *Stenotrophomonas* sp. WZN-1 which was only detected in the presence of strain A. This was interpreted as an interesting coincidence, yet the results were worthy of mentioning. The species was isolated for the first time from soil samples acquired from a waste recycling site in China. The microorganism could degrade polybrominated diphenyl ether compounds (PBFE), which were declared among organic pollutants in 2009. PBFE is a preferred compound in electronic equipment, furniture, plastic bottles, and textile materials due to its flame-retardant properties (Wu et al. 2017). The presence of *Stenotrophomonas* sp. WZN-1 strain implied mixing with litter specifically of plastic origin. *Arcticibacterium luteifluviistationis* was provided as another striking example. The species was identified in all strains, with the highest count number in the presence of strain B. Species were sampled from arctic sea water, and identification in Turkey was an interesting result (Li et al. 2017). Based on Li et al.'s findings, species could utilize esculin, the main ingredient of the horse chestnut. Hence results implied that manure, the main ingredient of compost, be stored in a horse-chestnut plantation area. The highest number of species in the presence of B strain was an expected result considering SGNH-type acetyl xylan esterases found in species. The enzyme was known to be effective in the degradation of xylan (Zhang et al. 2021).

Metagenome analyses, when evaluated with the results of composting experiments, provided solid explanations of the behavior of strains when inoculated in compost systems. Besides, interesting clues regarding the conditions of composting and storage. Metagenome results could also provide insight into the presence of pollutants when evaluated in detail.

Conclusion

Simultaneous evaluation of the results obtained from composting experiments and metagenome analyses led to the following conclusions:

Strains when added to compost systems dominated the entire compost microbiota.

The main factor establishing the dominance was the enzyme activity of strains. In other words, strains added in high amounts to compost systems could easily alter and/or enhance the degradation of organic matter.

Diversity of compost microbiota or count numbers obtained in any level (family, genera, strain) had second importance as long as high organic matter degradation could have been achieved as was shown in the presence of the C strain.

Enzyme activity was found to be among the definitive factors in the formation of the pathway leading to organic matter degradation. Degradation of organic matter in the presence of strain A depended entirely on cellulose degradation. On the other hand, organic matter degradation in the presence of the C strain followed an entirely different path. The presence of this variant route was validated via both composting experiments and metagenome analyses.

Degradation products of cellulose were favored by compost microbiota. On the other hand, xylan degradation was only effective as an enhancer in the transportation of microbiota members to cellulose along with the thrival of microbiota members with the ability to degrade xylan. In the presence of combined cellulase and xylanase activity, members of microbiota that could utilize xylan would thrive due to the change in preference of dominant strain.

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Author contributions All authors read and approved the final manuscript, including the names and order of authors. The final manuscript has been read and approved by all authors. Fadime Ozdemir Kocak and Levent Degirmenci designed the experiments, supervised the study, analyzed the results, and wrote the manuscript. Saadet Gizem Ertekin Tanır and Ayten Kumas Cetin carried out the experiments with help from Fadime Ozdemir Kocak and Levent Degirmenci.

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Data availability All data generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. The raw sequence reads data were submitted to NCBI Sequence Read Archive (SRA), and the study was registered in NCBI BioProject and BioSample database (SubmissionID: SUB12291991; BioProject ID: PRJNA903545). GenBank accession numbers of *Streptomyces* spp. are also received from NCBI.

Declarations

Competing interests The authors declare no competing interests.

Consent to participate Not applicable.

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