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Food Waste Composting with Wood Chips: Preliminary Analysis of the Bacterial and Fungal Communities

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Wood chips represent an important component in improving the composting of food waste by contributing to the optimization of the carbon/nitrogen ratio and providing porosity and aeration of the mass, which are determinant factors for proper aerobic fermentation and maturation processes carried out by microbial communities. This work evaluated the taxonomic complexity and functionality of the microbial communities associated with the composting process of three different w/w (wet weight) ratios of food waste and wood chips using traditional isolation methods and high throughput sequencing techniques (HTS). Results highlighted differences in the presence of specific taxa associated with certain ratios. The obtained results make a substantial contribution to understanding microbial dynamics during food waste composting.

1. Introduction

Disposal and treatment of food waste pose challenges, potentially leading to soil consumption, greenhouse gas emissions, foul odors, and leakage (Palaniveloo *et al*., 2020). Composting offers a solution to convert food waste into a valuable product; however, it is not straightforward to produce safe and quality compost since the starting material has physiochemical characteristics that hinder the aerobic degradation of the organic matter (Cerda *et al*., 2018). To overcome these technical problems, bulking agents were added to tune the balance between carbon and nitrogen and to allow proper aeration of the material. The most used bulking agents were woody residues (Li *et al.*, 2013) and biochar (Waqas *et al*., 2018). The choice of a suitable bulking agent and its quantity were among the most critical factors to consider before starting the process because high moisture, poor aeration, and inadequate pH levels can promote undesirable fermentation. Conversely, specific aerobic microbial taxa capable of degrading recalcitrant molecules, regulating pH levels, and promoting the production of humic substances are crucial. Since the presence of certain taxa is related to specific physiochemical parameters (Palaniveloo *et al*., 2020), detecting these taxa could give important information about compost quality or warn about the presence of unfavorable conditions. This study evaluated the microbial communities of three different weight-to-weight (w/w) ratios of food waste and wood chips (2:1; 3:1 and 5:1 w/w) using traditional isolation methods and high throughput sequencing (HTS) techniques. The aim was to determine the effects of different amounts of wood chips on food waste composting performance and on the microbial communities, leading to the development of a methodology capable of detecting specific taxa associated positively or negatively with the composting process and its maturity.

2. Materials and methods

2.1 Composting protocol and sampling design

Composting material included seasonal fruits and vegetables. Before composting, the material was shredded manually to reach a particle dimension of about 5-8 cm. The bulking agent was composed of woodchips. Composting was carried out using rotating composting bins of 180 L. Three different ratios of food waste to wood chips were studied based on a wet weight ratio (w/w): 2:1, 3:1, and 5:1. Composting process lasted 60 days (from 1/06/2023 to 31/07/2023) during which samples of 10 grams used for microbial isolation were taken from each composting bin thermophilic phase (day 4), cooling phase (day 20) and at the end of the maturation

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phase (day 60). Samples for metagenomic analysis were collected following temperature changes on ranges of 10 °C each during the active phase (until day 30), while every 15 days during the maturation stage (from day 31 to 60).

2.2 Chemical and Physical Analysis

Temperature was measured using a long-stem thermometer (Humboldt Mfg. Co., Range of –50 to 150°C, Stem length 20 cm). The moisture content was determined by drying at 105 °C for 24 h. The pH was measured in a 1:10 (w/v) water extract. Total organic carbon (TOC) and nitrogen (N) were determined following the standards UNI EN 15936:2012 (DIN, 2012) and UNI EN 16168:2012 (En, 2012) respectively. Organic matter content was assessed by determination of weight loss on ignition at 550 °C.

2.3 Microbial isolation

Fungi and bacteria were isolated from a suspension obtained by adding 10 g of fresh material to 90 mL of sterile saline solution (0.9% NaCl in distilled water). The suspension was shaken (150 rpm) at room temperature for 30 min. After that, a 10-fold serial dilution method was performed and 100 µL aliquots of each dilution were spread out in five replicates of Rose Bengal Agar and five of PCA Agar plates. Fungi and bacteria were allowed to grow for 72 and 24 h respectively at 30 °C (mesophilic and mature stage) or 45 °C (thermophilic stage). Results were expressed as colony-forming units (CFU) per milliliter of suspension. All different morphotypes identified were isolated and maintained in the same conditions as above and regularly transferred to fresh agar.

2.4 Molecular identification of the isolates

The sequence of ITS and 16S regions was used for fungi and bacteria identification respectively. For fungi, genomic DNA was extracted from the pure microbial cultures, using the kit “NucleoSpin Plant II” (Macherey–Nagel Gmbh & Co. Düren, Germany), according to manufacturer instructions. For bacteria, amplification was done directly by scrapping single colonies. The amplification reaction was carried out using primers ITS1 (5’-TCCGTAGGTGAAC CTGCGG-3’)/ITS4 (5’ -TCCTCCGCTTATTGATATGC-3’) for fungi and the 16s rRNA gene primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) for bacteria. Negative controls were used at each stage.
The PCR reaction mix comprised 12.5 µL of GoTaq® Hot Start Master Mix (Promega, Madison, WI, USA), 1 µL for each primer, 1 µL of Bovine Serum Albumine (ThermoFisher Scientific, Waltham, MA, USA), and 1 µL of template for a total volume of 25 µL. For bacterial isolates, the microliter of template was not added since DNA extraction was not performed. The presence of the amplicons was determined through electrophoresis on a gel with 1% of agarose and visualized by staining with Gel Red (Biotium). Amplicons were cleaned using the NucleoSpin Gel and PCR Clean-up Reagent kit (Macherey–Nagel; Düren, Germany), following the manufacturer's specifications, and quantified via “Quant-it Assays” (Invitrogen, Eugene, Oregon, USA). The suitably prepared samples were sent to Eurofins genomics (Luxemburg; http://www.eurofins.com) for Sanger sequencing. The sequences obtained were analyzed using the Bioedit Sequence Alignment Editor software and were compared with those present in the NCBI (National Center for Biotechnology Information) database using the NCBI Blast function (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.5 High Throughput Sequencing

For the HTS analysis, two replicates of DNA extraction (0.5 g of material each) were performed for each sample (for both fungi and bacteria). Total DNA was extracted using the kit “Nucleospin Soil” (Macherey–Nagel Gmbh & Co. Düren, Germany) according to the manufacturer’s instructions and then pooled together. The PCR reaction mix comprised 12.5 µL of GoTaq® Hot Start Master Mix (Promega, Madison, WI, USA), 1 µL for each primer, 1 µL of Bovine Serum Albumine (ThermoFisher Scientific, Waltham, MA, USA), and 1 µL of template for a total volume of 25 µL. Multiplexing PCR was carried out using a set of barcoded primers with a dual indexing primer targeting the ITS2-5.8S-ITS2 and 16S regions for fungi and bacteria respectively. Two replicates of PCR were conducted, and the products were pooled. Amplicons were purified using the MagJET NGS Cleanup (ThermoFisher Scientific, Waltham, MA, USA), quantified with the Qubit dsDNA HS assay kit, and pooled at equal concentrations for sequencing. Paired-end sequencing (2 × 300 bp) was carried out on an Illumina MiSeq sequencer by Fasteris (Genesupport SA, Plan-les-Ouates, Switzerland).

2.6 Bioinformatic analysis

The bioinformatics pipeline of (Morales-Rodríguez *et al*., 2021) was followed to decrease false assignments and cross-contamination, to perform quality filtration, trimming, merging, OTU clustering, and OTU identification.
Qiime2 (Bolyen *et al*., 2019) was used for metacommunity analysis of composition “ANCOM” (Mandal *et al*., 2015).

3. Results and discussion

3.1 Temperature and physiochemical characteristics of compost

After 24h from the beginning of the trial, the temperature of the material started to increase by reaching the peak after 3-4 days. After that, the material started cooling and reached the ambient temperature around the 20th day. Ratio 3:1 showed to have a stronger thermophilic phase compared to the others, with temperatures above 50 °C and maintaining the material above 40°C for a week (Figure 1). The amount of humidity, was particularly high in the ratio 5:1 (76%) due to smaller amount of bulking agent while was considered acceptable in the ratios 2:1 and 3:1. Nonetheless, at the end of the 60th day, humidity was below the limit of the Italian national regulation in all three cases (Dlgs. 75/2010, published in G.U. n.126 26/05/2010). The C/N ratio was low for all three trials, due to the high amount of nitrogen in food waste (Wang & Zeng, 2018). This condition caused the loss of precious nitrogen probably through leachate in the 5:1 ratio as its C/N ratio increased sensitively into mature compost and its (total nitrogen) N tot decreased by 28%. Regarding trials 2:1 and 3:1 C/N ratio remained constant, and a lower amount of nitrogen was lost thanks to better starting conditions. The results of the physiochemical analysis of the starting material and the mature compost are displayed in Table 1.

Table 1: Results of the physiochemical analysis of the three compost mixes at the beginning (day 0) and at the end of composting cycle (day 60). The expressed ratios are between agri-food waste and wood chips.

Index	U.M	2:1 (0)	3:1 (0)	5:1 (0)	2:1 (60)	3:1 (60)	5:1 (60)
pH		4.5	3.9	3.9	6.2	6.8	7.6
Humidity	%	53	70	76	41	45	46
TOC	%ss	35	32	29	31	27	35
N tot	%ss	1.9	2.1	2.1	1.5	1.6	1.5
C/N		18	16	13	20	16	23

3.2 Microbial isolates

The colony-forming units (CFU) count revealed a notable prevalence of bacteria over fungal populations during the composting period (Figure 2). The thermophilic phase was characterized by a generalized low microbial load. This occurred because high temperatures created a hostile environment for most of the microorganisms, favoring the proliferation of specific bacteria, and decreasing the other taxa (Ryckeboer *et al*., 2003). After the thermophilic phase, where the degradation of recalcitrant molecules such as lignin and hemicellulose took place (Zhu *et al*., 2021), the decrease in temperatures created a favorable environment for the growth of fungi and other bacterial species which resulted in a higher CFU/ml count. The higher presence of lignocellulosic bulking agents in the ratios of 2:1 and 3:1 appeared to promote the growth of fungi into mature compost, while in the ratio of 5:1 a lesser presence of fungi was evident. Notably, the 3:1 ratio exhibits a consistent increase in both fungal and bacterial presence over time. These trends may be attributed to the unique capacity of fungi to degrade lignocellulosic molecules, to the superior physiochemical characteristics of the compost mix 3:1, and to its more intense thermophilic phase, which helps the effective degradation of complex molecules (Turan & Ergun, 2008). The overall increase of the microbial biomass in mature compost matches with the findings of Li *et al.*, (2013) and Pot *et al.*, (2021) which demonstrated the positive correlation between maturity and microbial diversity.

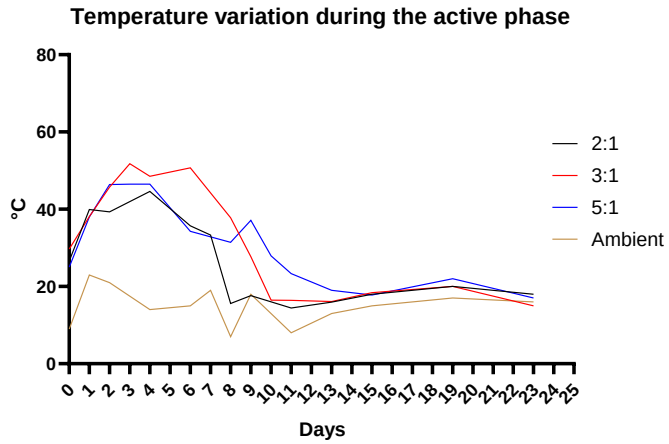

Figure 1: Temperature trend in the first 25 days of the three compost mixes and of the ambient temperature.

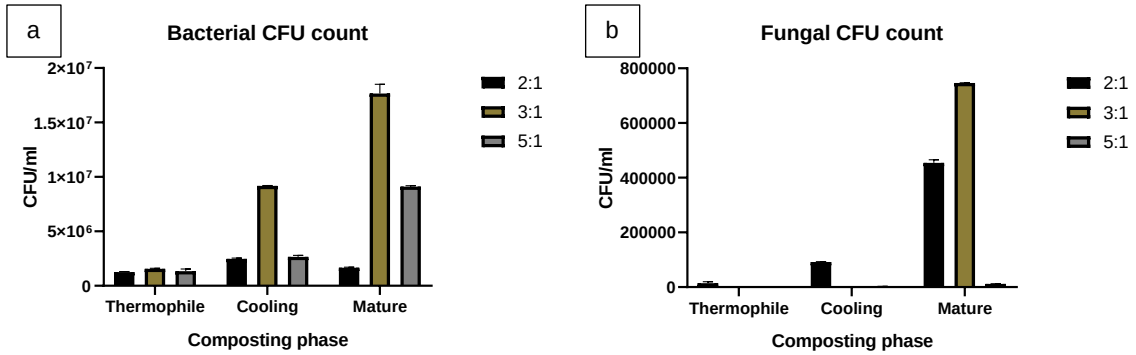

Figure 2: Bacterial (a) and fungal (b) CFU count for each composting ratio measured during the three main phases of the process.

The taxa isolated from single colonies (Table 2) belonged to nine different bacterial genera including members of the *Bacillus amyloliquefaciens* group, classified as Plant Growth Promoting Bacteria (PGPB) (Ngalimat *et al*., 2021). Among members of the genus *Pseudomonas*. *Pseudomonas mucidolens* which is capable of degrading low-density polyethylene (Krishnaswamy *et al*., 2022), and *Pseudomonas aeruginosa* which can potentially pose a risk to human health (Bassetti *et al*., 2018) together with the bacteria *Klebsiella pneumoniae* (Li *et al*., 2014). Regarding fungal diversity twelve genera were isolated, many of them described as capable of degrading cellulose, lignin, and hemicellulose such as *Acaulium* spp. (He *et al*., 2022) and *Bjerkhandera* spp. (Robledo-Mahón *et al*., 2020) or to regulate the level of organic acids in compost like *Pichia kudriavzevii* (Nakasaki & Hirai, 2017). There are several explanations for the existence of pathogens in compost, including secondary colonization from the surrounding environment (Palaniveloo *et al*., 2020); yet, it's also likely that they were present in the starting material and survived the entire composting process.. Interestingly, the ratio of 3:1 did not show the presence of bacterial pathogens; possibly, its stronger thermophilic phase contributed to the removal of the other hazardous organisms.

Table 2: fungal and bacterial taxa isolated from the three different compost mixtures, where "x" means presence. The letter into the brackets indicate the phase of isolation: T=thermophile, C=cooling and M=mature.

Fungal taxon (Phase)	2:1	3:1	5:1	Bacterial taxon (Phase)	2:1	3:1	5:1
Aspergillus flavus (T,C,M)	x	x	x	*Microbacterium* spp. *(T,C)*	x	x	
Trichurus spiralis (T,C,M)	x			*Klebsiella pneumoniae (T,C)*	x		
Lichteimia corymbifera (T,C)	x	x	x	*Pseudomonas aeruginosa (C,M)*	x		
Geotrichum candidum (T,C,M)	x	x	x	*Brucella gallinifaecis (C,M)*	x		
Parascedosporium putredinis (C,M)	x		x	*Kestersia gyiorum (C)*	x	x	x
Aspergillus fumigatus (C,M)	x		x	*Bacillus subtilis (T,C,M)*	x	x	x
Cladosporium spp. *(C,M)*	x			*Lysinibacillus pakistanensis (C)*	x	x	
Pichia kudriavzevii (T,C)	x		x	*Bacillus amyloliquefaciens* group *(T,C,M)*	x	x	x
Bjerkandera adusta (C)			x	*Kurthia gibsoni (T,C)*	x	x	
Acaulium album (M)			x	*Pseudomonas mucidolens (M)*	x		
Penicillium polonicum (M)	x			*Pseudomonas composti (M)*		x	
Lomentospora prolificans (T,C,M)	x	x	x	*Cellulosimicrobium composti (M)*	x		
Mucor fragilis (M)		x					

3.3 Microbial community characteristics

ANCOM analysis (Mandal *et al*., 2015) allowed the identification of specific taxa in compost mixes. For fungi, *Barnettozyma californica* was found particularly abundant in the ratio of 5:1 where N amount was higher, and its loss was greater during composting. This yeast can degrade edible oil thanks to lipase production (Ciafardini *et al*., 2006) and has an interesting ammonium reduction (Falih & Wainwright, 1995) and aerobic denitrification

capability (Fang *et al*., 2021) which could explain its more abundant presence in the ratio of 5:1. Among bacteria, the ratio of 5:1 was particularly enriched by anaerobic bacterial genera such as *Bacteroides* (Wexler, 2007) and *Phascolarctobacterium* (Wu *et al*., 2017) which are commonly found inhabiting the human body but also by *Advenella* sp. which included some species associated with N-rich environments such as slurry wastewaters (Matsuoka *et al*., 2012) and others that can harbor plant growth promoting activities like phytase production (Singh *et al*., 2014). *Bordetella* and *Delftia* instead, were more present in the ratio of 2:1. Genus *Bordetella* includes several species, some of them well studied because are causing diseases in humans such as *B. pertussis* and others isolated from environmental samples which are less known (Kamanova, 2020). *Delftia* spp. is known to be particularly useful for bioremediation thanks to its capacity to store heavy metals (Benndorf & Babel, 2002).

4. Conclusions

The findings indicated that the use of wood chips at a 3:1 ratio demonstrated superior chemical and physical properties, resulting in a more pronounced thermophilic leading to the production of a safer product. Conversely, ratios of 2:1 and 5:1 exhibited suboptimal performance. Microbiological analysis carried out in this study demonstrated the predominance of the bacterial population over the fungal population and the importance of compost as a source of potentially useful microorganisms such as *Pseudomonas mucidolens* and *Pichia kudriavzevii* which could serve as valuable microbial starters in future applications to enhance composting. Metabarconding allowed the detection of specific microorganisms associated with suboptimal composting parameters such as *Bacteroides* spp., thriving in anaerobic conditions, and *Barnettozyma californica*, prevalent in nitrogen-rich environments. The results of this study contributed to a more efficient use of wood chips in food waste composting and deepen the knowledge regarding microbial communities involved in the composting process.

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