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ORIGINAL ARTICLE

Characterization of bacterial communities responsible for bioplastics degradation during the thermophilic and the maturation phases of composting

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Abstract
Bioplastic waste may play a key role in shaping the microbial communities in composting environment. The present study aims to determine the possible influence of bioplastics on microbial structure of aerobic organic waste treatments and to assess the evolution of microbial community during thermophilic and maturation phases of composting, comparing microorganisms in compost and attached on bioplastics surface. Composting process was simulated in a 2-month lab-scale test. Mater-Bi®, PBAT, PLA and LDPE were considered as benchmark. A qualitative screening was done with DGGE, and then the bacterial community profile was disclosed with 16S rRNA amplicon analysis. The 16S rRNA profile in bioplastics-associated communities was found to largely differed from that of the initial compost (positive control). Moreover, as expected, during the thermophilic phase there was a prevalence of better heat-resistant phyla compared to the mesophilic phase. Some specialists were found to be plastic nature dependent, in particular *Streptomyces*, *Pseudomonas*, *Aeribacillus*, *Schlegellela* and *Cohnella* were among the most abundant genera. Finally, to select bacterial species capable of growing on the tested bioplastics, an enrichment method was applied. The method disclosed the possibility to culture some specific bioplastics degrading species, opening the frontiers for bioaugmentation practice in industrial composting.

Keywords Bioplastic degradation · Composting · Biofilm · Bacterial community · Enrichment culture

Introduction

In accordance with the European Bioplastics Agency, bioplastics are divided into three categories: biobased bioplastics, which are derived from renewable resources; biodegradable bioplastics, degradable by microorganisms; bioplastics which feat both the characteristics. Some commonly used bioplastics are biodegradable through composting. Therefore, they are called compostable bioplastics and can be biobased or derived from fossil fuel. Even though compostable bioplastics represent just a small part of the global plastic production, their increase on the market will require further research to improve the management in composting plants for organic waste treatment. Indeed, since the existing plants processing the organic waste were not designed to treat also bioplastics, they may not be totally effective in the management and deterioration of these materials [1]. Furthermore, it is fair to highlight that data from the Italian Composting and Biogas Association, named CIC (*Consorzio Italiano Compostatori*) revealed that part of the plastics arriving to composting plants are not biodegradable (e.g., low-density polyethylene) [2]. During degradation, the polymer is first biodeteriorated and depolymerized by a synergic action of physical and biological agents; then the smaller monomers are assimilated by microorganisms and mineralized [3, 4]. Thus, the microbial community plays a fundamental role in plastics degradation, as polymers are potential substrates for heterogeneous microorganisms. Overall, microbial communities associated to plastics are responsible for the degradation and catabolism with amylolytic, hydrolytic or lipolytic activity thanks to intracellular

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and extracellular specific enzymes [5, 6]. It is fair to mention that microbial diversity is one of the most influential factors in bioplastics degradation, together with environmental and operating conditions [7]. Many studies were performed in the last decade to identify microorganisms, both aerobes and anaerobes, capable to assimilate both natural and synthetic plastics, mainly in soil and in aquatic environments [8, 9]. In the field of plastic litter in marine environment, it was introduced the term "Plastisphere" to describe the diverse microbial community attached to plastic surfaces and distinct from the surroundings [10]. Even though compost is less studied than aquatic environment, some recent studies identified different bacteria, fungi, archaebacteria and lower eukaryotes capable to enhance bioplastics degradation in composting [8, 11, 12]. However, there is a need for further investigation to understand the effects of microorganism species, population, and enzyme specificity on the biodegradation process of bioplastic waste [7]. Indeed, bioplastic waste may play a key role in shaping the microbial communities in composting environment, as well as it has been disclosed for plastic litter in marine environment [10, 13].

The main goal of research about bioplastics degradation is to improve their management in organic waste treatments. Until now, most of the studies focused on chemical and physical degradation of bioplastics, with the aim to improve the operating conditions of organic waste treatments [3, 14, 15]. On the contrary, in this field, there are fewer biological studies, even though new findings on bacteria and other microorganisms capable of degrading bioplastics may improve their management in organic waste treatments. Therefore, this study aims (i) to determine the possible influence of bioplastics on microbial structure of aerobic organic waste treatments; (ii) to assess the evolution of microbial community during thermophilic and maturation phases of composting, comparing microorganisms in compost and attached on bioplastics surface; (iii) to select bacterial species capable of growing on the tested bioplastics, applying an enrichment method. The present study is performed with a lab-scale composting test. However, differently from other research reported in the literature, this study tries to come closer to the conditions of industrial composting process. Indeed, it is fair to underline that research studies about bioplastics in composting generally follow the operational conditions of the main standards (EN 13432:2000; ISO 20200:2015; ASTM D5338:2011 [16–18]). These standards require a minimum period of 45 days at temperatures not lower than 58 °C, which are not strictly representative of highly variable industrial composting conditions. To select the operational conditions of the present study, an overview about composting treatments in Europe, provided by the European Commission, was considered. The mean length of the thermophilic phase in these treatment plants is 2–3 weeks, while the maturation phase varies from a minimum of 2 weeks to few months [19]. Starting from this overview, the thermophilic and the maturation phase in the lab-scale test last for 20 days and 40 days, respectively. Various bioplastics commonly used for film and rigid manufacturing are tested (Mater-Bi, PLA and PBAT compared to LDPE). Our innovative approach allowed to make detailed observation on bacterial community differentiation and specialization in composting conditions closer to the real word ones.

Materials and methods

Tested materials

Samples of the following bioplastics were submitted to a composting test: starch-based Mater-Bi® (MB), Polybutylene Terephatlate (PBAT) and PLA. Mater-Bi® is widely employed to produce bags for organic waste collection. MB bags, bought in Italian supermarket, were manually cut (thickness = 50 µm, size = 5 × 5 cm). The material is composed of starch (20%), polybutylene adipate terephthalate (PBAT) (70%) and some additives (10%) [20]. Moreover, PBAT is not a biobased bioplastic, but it derived from fossil fuel. As this is the biggest part of Mater-Bi®, pure PBAT was separately tested to better investigate its degradation process. Ecoflex® F Blend C1200 PBAT (thickness = 90 µm, size = 5 × 5 cm) was supplied by the producer company BASF. The third material was PLA. However, differently from MB and PBAT, it was tested in rigid form, in order to simulate the degradation of some thick products made of PLA and widely diffused on the market, such as single use cutleries, dishes and glasses. PLA was supplied by NatureWorks LLC (USA): PLA98.6/1.4 (L/D-lactide) is a grade for the extrusion of films. Pellets are dried in vacuum oven overnight at 60 °C and processed using a press model 4122CE manufactured by Carver, with circle shape (thickness = 500 µm, ø = 5 cm). LDPE in film form was introduced in the test as a negative benchmark for its low degradation efficiency. LDPE film ET311350/2 was bought from Goodfellow company (UK) (thickness is = 250 µm, size = 5 × 5 cm).

Experimental setup

Composting test was carried out at lab scale and in accordance with the modified guidelines EN ISO 14855-2:2018 [21]. A 3-month stable compost (IR5 5.6 mg O2/g TS) from green and agricultural waste was provided by Ipalle industrial composting plant, located in Froyennes (BE), together with the chemico-physical analyses carried out by Liège University (BE). Humidity, C/N and pH of compost were 47%, 15 and 7.5, respectively. In accordance with ISO 14855-2:2018, test aiming to study microbial community

evolution, shall use stable compost as inoculum of the aerobic biomass to provide sufficient diversity of microorganisms and enhance the degradation process of the tested materials [21]. Indeed, the test lasted for 60 days (d) with a thermophilic phase of 20 days at 58 ± 2 °C followed by a maturation phase under mesophilic conditions (37 ± 2 °C). Regarding the temperature profile, it is fair to report that when moving from thermophilic to mesophilic phase, the temperature was not suddenly changed, in order to allow bacteria to adapt to the new temperature conditions. Temperature profile is shown in Figure S1.

10 g of plastic was completely mixed in 350 g of stable compost (concentration 2.9%). Three replicates for each tested material were prepared. Moreover, three replicates with compost only were prepared as positive control. In Table 1, the experimental runs with the description of the

Table 1 Experimental runs with description of test conditions

Positive control: reactors with compost only			
Blank1 start	Compost immediately after harvesting from the plant		
Blank2 start			
Blank3 start			
Blank1T	Compost from blank reactor after 20 days at 58 °C		
Blank2T			
Blank3T			
Blank1M	Compost from blank reactor at the end of the test at the end of test (20 days at 58 °C + 40 days 37 °C)		
Blank2M			
Blank3M			
Experimental test: reactors with compost and bioplastics			
Bioplastics—20 days		Bioplastics—end of the test	
MB1T pl	MB after 20 days at 58 °C	MB1M pl	MB at the end of test (20 days at 58 °C + 40 days 37 °C)
MB2T pl		MB2M pl	
MB3T pl		MB3M pl	
PBAT1T pl	PBAT after 20 days at 58 °C	PBAT1M pl	PBAT at the end of test (20 days at 58 °C + 40 days 37 °C)
PBAT2Ti pl		PBAT2M pl	
PBAT3T pl		PBAT3M pl	
PLA1T pl	PLA after 20 days at 58 °C	PLA1M pl	PLA at the end of test (20 days at 58 °C + 40 days 37 °C)
PLA2T pl		PLA2M pl	
PLA3T pl		PLA3M pl	
LDPE1T pl	LDPE after 20 days at 58 °C	LDPE1M pl	LDPE at the end of test (20 days at 58 °C + 40 days 37 °C)
LDPE2T pl		LDPE2M pl	
LDPE3T pl		LDPE3M pl	
Compost—20 days		Compost—end of the test	
MB1T co	Compost in MB reactor, collected near the piece after 20 days at 58 °C	MB1M co	Compost in MB reactor, collected near the piece at the end of the test
MB2T co		MB2M co	
MB3T co		MB3M co	
PBAT1T co	Compost in PBAT reactor, collected near the piece after 20 days at 58 °C	PBAT1M co	Compost in PBAT reactor, collected near the piece at the end of the test at the end of test (20 days at 58 °C + 40 days 37 °C)
PBAT2T co		PBAT2M co	
PBAT3T co		PBAT3M co	
PLA1T co	Compost in PLA reactor, collected near the piece after 20 days at 58 °C	PLA1M co	Compost in PLA reactor, collected near the piece at the end of the test at the end of test (20 days at 58 °C + 40 days 37 °C)
PLA2T co		PLA2M co	
PLA3T co		PLA3M co	
LDPE1T co	Compost in LDPE reactor, collected near the piece after 20 days at 58 °C	LDPE1M co	Compost in LDPE reactor, collected near the piece at the end of the test at the end of test (20 days at 58 °C + 40 days 37 °C)
LDPE2T co		LDPE2M co	
LDPE3T co		LDPE3M co	

The acronyms in samples names refer to: type of bioplastic tested: *MB* Mater-Bi, *PBAT* polyethylene adipate terephthalate, *PLA* polylactic acid, *LDPE* low-density polyethylene. Temperature conditions: *start* before the test, *T* at the end of the thermophilic phase of the test, *M* at the end of the maturation phase of the test. Where the sample is collected: *pl* sample collected on plastic surface, *co* sample collected from compost in the surrounding of plastic piece, *blank* sample collected in the positive control with compost only

test conditions and of the acronyms used for samples names are reported.

The composting test was carried out in glass reactors, cylindrically shaped, with a volume of 5 l, manufactured by Pierre E. bvba (Vilvoorde, BE). The reactors were connected with a system provided by Wetlands Biosciences sprl (Louvain-la-Neuve, BE). This system allowed the automatic control of the air flow through the reactors. Indeed, the air flow was humidified by passing through distilled water in a glass bottle, of which each reactor was provided, keeping the samples humidity in the constant range required by the standard EN ISO 14855-2:2018 (50–55%). The glass reactors were then placed into two Binder BD400 incubators to control the temperature conditions. In Figure S2 of the Supplementary Materials, the equipment is reported.

Reactors were opened once a week: pH and humidity were checked: pH of the samples stayed in the range 7–7.5 during the 60 days of the test. The humidity was measured with total solids (TS) content analysis, in accordance with the standard ISO 11465 [22]. It was manually adjusted during the test to be kept in the required range. Moreover, the sample was mixed to ensure a proper homogenization of the plastic material within compost. Finally, before mixing, the samples were weighted to assess that the 350 g of stable compost in the reactor did not decrease.

Characterization of the tested materials

Samples of both compost and plastic fragments were recovered at the end of the thermophilic phase and at the end of the test, to carry out the analyses of the microbial communities. Moreover, the residues of the tested materials were submitted to chemical and physical analysis to observe the degradation after composting. First, the pieces were weighted to obtain an experimental measure of the weight loss after the composting test. ThermoGravimetric Analysis (TGA) was performed to observe the characteristic temperatures of the materials before and after the degradation. FTIR allows to monitor the degradation of the chemical structure of the materials, and SEM analysis provides a highly precise overview of the morphological changes. The methodologies and the results of this preliminary part of the test are fully described in Ruggero et al. 2021 [23].

Denaturing gradient gel electrophoresis

The bacterial community structure and diversity were preliminarily studied by DGGE in compost (positive control and reactors with test material) and bioplastic pieces. Samples of compost (positive control and reactors with tested materials), of bioplastics and of LDPE in triplicates were recovered and directly processed after the thermophilic phase and at the end of the test. Therefore, a total amount of 57 samples was processed. All the procedures are described in the Supplementary Materials. The results were evaluated by comparing samples on a single gel (difference of composting conditions for one plastic type), and between gels (difference between plastics) thanks to the blank which served as reference among gels. This comparison allowed a preliminary qualitative observation about the presence of eventual common bands and peculiar bands of each sample.

16S rRNA amplicon sequencing

The 16S rRNA amplicon analysis was made by Novogene, with the following procedure (reported in Novogene 16S Analysis Report of X204SC20050178-Z01-F003). Total DNA from samples was extracted using CTAB/SDS method: cetyl trimethylammonium bromide (CTAB) is a cationic detergent that releases the cellular inner components and promotes the separation of proteins and polysaccharides from nucleic acids, while sodium dodecyl sulfate-based (SDS) buffers are used for the lysis step [24]. DNA concentration and purity were monitored on 1% agarose gel. According to the concentration, DNA was diluted to 1 ng/μl using sterile water. 16S rRNA genes of distinct regions (16SV4/16SV3/16SV3-V4/16SV4-V5, Arc V4) were amplified using specific primers, e.g., 16S V4: 515F (5′-GTGCCA GCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVH-HHTWTCTAAT-3′). All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). The PCR cycle was as follows: 30 s at 98 °C, and then the first cycle was carried out 35 times using a denaturation temperature of 98 °C for 10 s, a hybridization temperature of 61 °C for 30 s (decreasing by 0.5 °C at each cycle) and an elongation temperature of 72 °C for 15 s. The PCR was ended by heating at 72 °C for 7 min. The same volume of loading buffer (contained SYBR green) was mixed with PCR products and loaded on 2% agarose gel for detection: samples with a bright distinct band between 400 and 450 bp were chosen for further experiments.

PCR products of the triplicates were mixed in equidensity ratios. Then, mixture PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using NEBNext® UltraTM DNA Library Pre-kit for Illumina, following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina platform and 250 bp paired-end reads were generated.

Sequences analysis was performed by Uparse software. Sequences with ≥ 97% similarity were assigned to the same Operational Taxonomic Units (OTUs). For each representative sequence, the Greengene database was used based on

Ribosomal Database Project (RDP) classifier algorithm to annotate taxonomic information.

Importantly, in order to study phylogenetic relationship of different OTUs, and the difference of the dominant species in the groups, multiple sequence alignment was conducted using the MUSCLE software (Version 3.8.31).

The sequencing quality was verified using the rarefaction curves using the PAST software. OTUs abundance information was normalized using Limma RGui package (15,090 read counts). To evaluate the microbial community diversity, Venn diagrams were performed using Venn Diagram RGui package using OTU presence/absence to assess the distribution into the different conditions. Alpha-diversity indices (OTU richness and the Shannon index) were calculated on the rarefied data using PAST3 software and the beta-diversity was assessed using multivariate analysis by the PERMANOVA test (vegan RGui package) [25]. This analysis tested the factor significance (type, plastic, place) using the Bray–Curtis dissimilarity with 10,000 permutations. A heatmap was performed with 34 OTUs for thermophilic conditions and 103 OTUs for the mesophilic conditions, showing that it was significantly affected by the different conditions. These OTUs were defined using a nbGLM (negative binomial distribution and Generalized Linear Model) revised by 1000 resampling iterations of the residual variance. The nbGLM is a deviance analysis performed using the mvabund RGui package [26]. Two RGs were defined with a cluster dendrogram using the Euclidean distance and an average clustering (vegan RGui package).

Enrichment cultures

Three 2 × 2 cm pieces of each bioplastic type and of LDPE were recovered, at the end of both thermophilic and maturation phase, with the purpose to isolate bacterial species specialized in plastic degradation. One piece of the material was taken from each reactor (three reactors per tested material) and doubly rinsed in PBS to detached compost and bacteria not strongly adherent to the plastic surface. The plastic samples were added into 50 ml polycarbonate Falcon® tubes filled with 15 ml low carbon source medium (0.2% ammonium sulfate, 0.05% yeast extract and 1% trace elements (0.1% MgSO4.7H2O, 0.1% FeSO4.7H2O, 0.01% ZnSO4.7H2O, 0.01% CuSO4.5H2O and 0.01% MnSO4.5H2O)) in 20 mM (N-morpholino) propane sulfonic acid (MOPS) pH 8; adapted from [27] and previously described [28]. The enrichment cultures were carried out by an incubation at 58 °C, for those pieces taken at the end of the thermophilic phase, and at 37 °C, for those collected at the end of the test (after the maturation phase).

Bioplastic and LDPE residues were recovered after 2 months of incubation and were submitted to FTIR and SEM, to observe the degradation process. The equipment used for FTIR and SEM analysis is described in the Supplementary Materials.

Bacterial isolations were also performed on the recovered pieces. First, bacteria were directly isolated from the plastic surface with the following protocol: plastic pieces were cut in millimetric fragments and transferred into a 50 ml tube Falcon® with 25 ml of PBS, previously filtered with a 0.2 µm pore size. Then, the steps were as follows: soft sonication on ice for 10 min to ensure a smooth released, vortex for few seconds, horizontal shaking for 20 min and finally vertical sedimentation and room temperature for 10–15 min. Petri dishes were prepared with Lysogeny Broth (LB) medium and 100 µm of diluted solutions (three 1:10 dilutions in water) were plated. Petri dishes were incubated at 37 °C and 58 °C for a period of 48 h (for the first and second dilutions) and 72 h (for the third dilution).To isolate the bacteria, each individual colony was collected with a bacteriological loop and striped into new LB plates. DNA was extracted from all these individual colonies using the QIAamp DNA Mini kit Qiagen, dedicated to isolation of genomic, mitochondrial, bacterial, parasite or viral DNA. DNA was purified following the manufacturer's instructions. After measuring the DNA concentration with BioSpec-nanomanufactured by Shimadzu Biotech, it was diluted at 2 ng/µl to allow the further steps of the procedure. Touchdown PCR amplification of 500 bp fragments of the 16S rRNA gene was carried out using primers: 534R (5′-ATTACCGCGGCTGCTGG-3′) and F8 (5′-TTTCATAATATGTGCTACGCAACCTA-3′). The PCR cycle was identical as the one described for the DGGE and PCR products were purified with AmpliClean™ Magnetic Bead PCR Clean-up kit in accordance with the instruction of manufacturer. 16S rRNA amplicon analysis was performed with Beckman Coulter CEQ-8000; the data were elaborated with Sequencing Analysis software and a database search was carried out using BLAST programme (NCBI, Maryland, USA) to identify the microorganisms at species and strains levels.

Results

Bacterial community structure

At the end of composting test, the tested materials displayed different levels of degradation. In particular, while MB showed large bacterial colonization, deep erosion signs and a significative weight loss (45 ± 5%), the other bioplastics displayed less evident signs of degradation, as well as a much lower weight loss (8 ± 1.6% PBAT and 3 ± 0.7% PLA). Finally, LDPE was almost undegraded (2% weight loss) and bacterial colonization on the plastic surface was negligible. These preliminary results, widely discussed in Ruggero et al. 2021 [23], delineated a correlation

between the activity of the microbial community studied and the effective degradation of the tested materials. The analyses of the bacterial communities were performed on the different samples (blank 'B', plastics 'Pl' namely MB-PBAT-PLA-LDPE and compost 'Co'), and on samples at different times of the composting process: at the launch of the test (Start, 0), at the end of the thermophilic phase (Thermo, 20 days), at the end of the maturation or mesophilic phase (Meso, 60 days). It is fair to mention that the species accumulation boxplot reports enough numerosity of the samples, which ensures good quality results. Moreover, the rarefaction curves of the data set generally tended to an asymptotic plateau, thus the species biodiversity is well represented (Figure S3a and b).

Preliminary observations about the community were extrapolated from the non-metric multidimensional scaling (nMDS) profile (Fig. 1).

The nMDS profile discriminated compost from plastics-associated communities, as well as thermophilic from mesophilic communities. The microbial community in harvested compost (blank start) was found to be highly different than after test incubation. The other samples of compost are all grouped together (color purple). Plastics under mesophilic conditions are divided into different groups, based on the type; on the contrary, plastics under thermophilic conditions are all into the same group (color yellow).

An equilibrium in the dominance of the species was generally observed from the equitability index (Fig. 2a and b).

LDPE under thermophilic conditions is an exception, showing the presence of dominant species in the communities. The communities peculiar of compost are characterized by a higher biodiversity of species than the communities developed on plastic samples, as shown by the richness index. Moreover, from the richness index, it emerged that the mesophilic communities (Fig. 2c) had a higher number of species than the thermophilic communities (Fig. 2d), both in compost and in plastics.

A common core about 177 (47%) and 195 (57%) OTUs for thermophilic and mesophilic conditions were displayed by the Venn diagrams (Fig. 3a and b). LDPE and PLA had a larger number of unique OTUs than MB and PBAT. 9.5 and 7.4% of thermophilic OTUs were unique to LDPE and PLA, respectively. The percentages were slightly lower under mesophilic temperature, with 7.3 and 7% of OTUs unique to LDPE and PLA, respectively. 4 and 3.7% of thermophilic OTUs were present uniquely in MB and PBAT. The percentages were lower for mesophilic OTUs, corresponding to 0.6 and 1.7% for MB and PBAT, respectively. The same common core was found in the preliminary qualitative screening provided by DGGE (Figure S4).

The 16S rRNA amplicon analysis revealed that the microbial communities were mainly composed of *Firmicutes*, *Actinobacteria* and *Proteobacteria*, the sum of all three reaching a minimum of 58.8% of the total community (communities on MB, mesophilic conditions). Bar graphs of phyla for each plastic type and compost were elaborated: in Fig. 4, it is reported the bar graph of the communities peculiar of Mater-Bi®, accounting samples recovered both from compost and from the bioplastic pieces.

The most abundant phylum in all samples was *Firmicutes*, except for the bioplastics-associated population in mesophilic conditions, where *Proteobacteria* represented 37–49% of the population. Compost-associated community had a balanced distribution among the major phyla (*Proteobacteria* 20–24%, *Actinobacteria* 20%, *Firmicutes* 25–35%), independently of the incubation conditions, except for 'blank meso' which had more *Bacteroidetes* and *Chloroflexi* (respectively, 15 and 11%). Conversely, the communities associated to bioplastics were a little more heterogeneous with a marked difference between thermophilic and mesophilic conditions: the

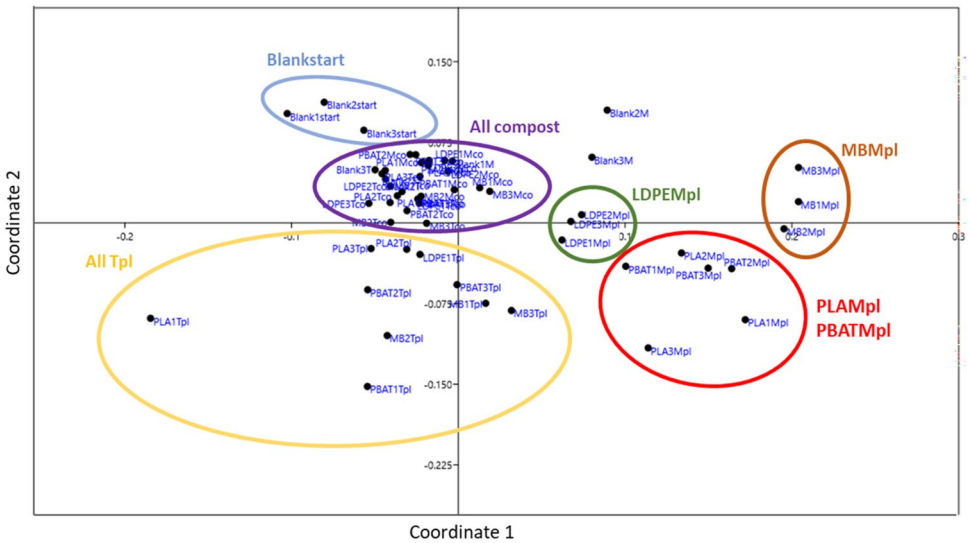

Fig. 1 nMDS profile of the pairwise community dissimilarity (Bray–Curtis) indices of the 16S rRNA amplicon sequencing. The samples from harvested compost before the test (blank start: blue circle) are well distinguishable; the compost samples from bioreactors during the test are all groupable in the purple circle. Plastics composted under thermophilic conditions stay all in the yellow group, while plastics under mesophilic conditions are groupable in three circles: red contains PLA and PBAT; green LDPE and brown MB

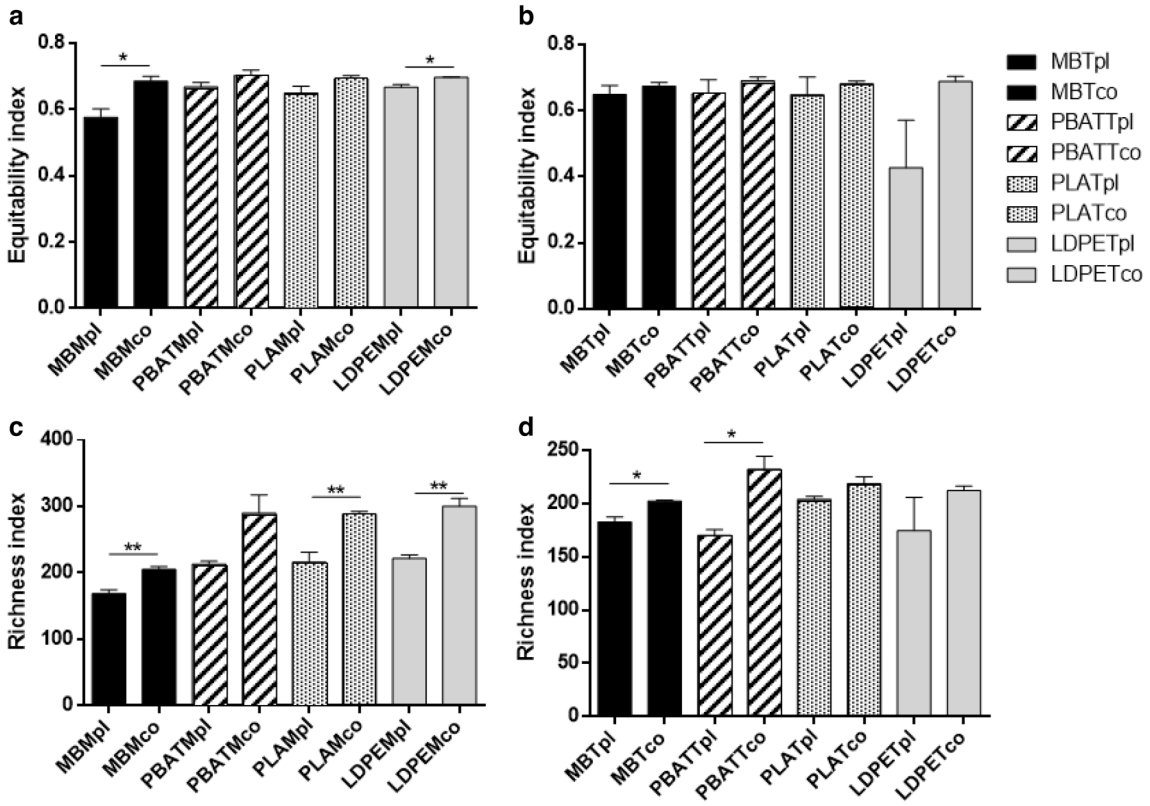

Fig. 2 **a** and **b** Equitability index (or Shannon) and **c** and **d** OTU richness obtained from 16S rRNA amplicon sequencing for samples types (plastics and compost under mesophilic and thermophilic conditions). *T*-test; $*P<0.05$; $**P<0.01$; $***P<0.001$

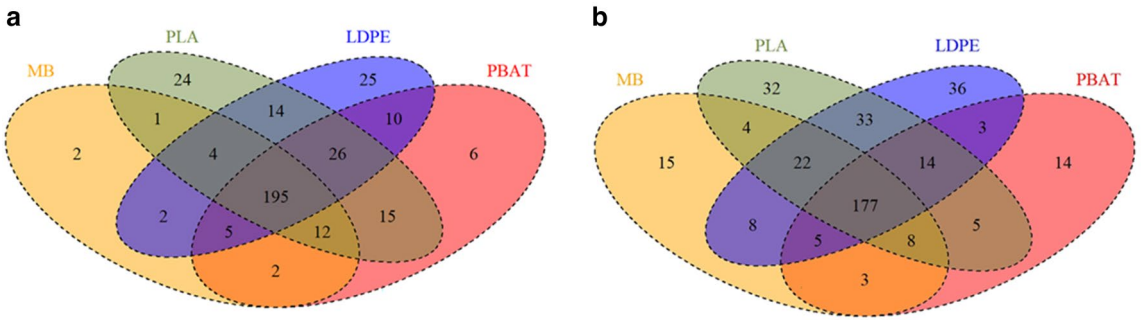

Fig. 3 Venn diagrams showing overlap of bacterial OTUs for the different tested materials: **a** after the maturation phase in mesophilic conditions, **b** after the thermophilic phase

community composition displayed a shift with an increase of *Bacteroidetes* abundance, reaching 10 to 23% of the population, and of *Proteobacteria* especially with the mesophilic bioplastics.

The heat maps (Fig. 5, S5 and S6) represent the relative abundance of genera in each compost and bioplastic sample, enabling to highlight the most abundant genera and the general trends.

Sphaerobacter, *Hydrogenispora*, *Planifilum* and *Tuberibacillus* were more present in compost, with, respectively, and average abundance of 4.88%, 2.79%, 1.68% and 1.28% in compost samples (including the blanks). The abundance of specific genera was associated to the composting conditions, i.e., the mesophilic and the thermophilic phases. *Thermopolyspora*, *Cohnella* and *Thermocrispum* (*Actinobacteria*), *Paenisporosarcina*, *Bacillus and Geobacillus* (*Firmicutes*), were found especially in thermophilic communities of bioplastic samples (Figs. 5 and S5). During the maturation phase, the phylum of *Proteobacteria* was largely predominant: in particular, *Pigmetiphaga*, *Steroidobacter*,

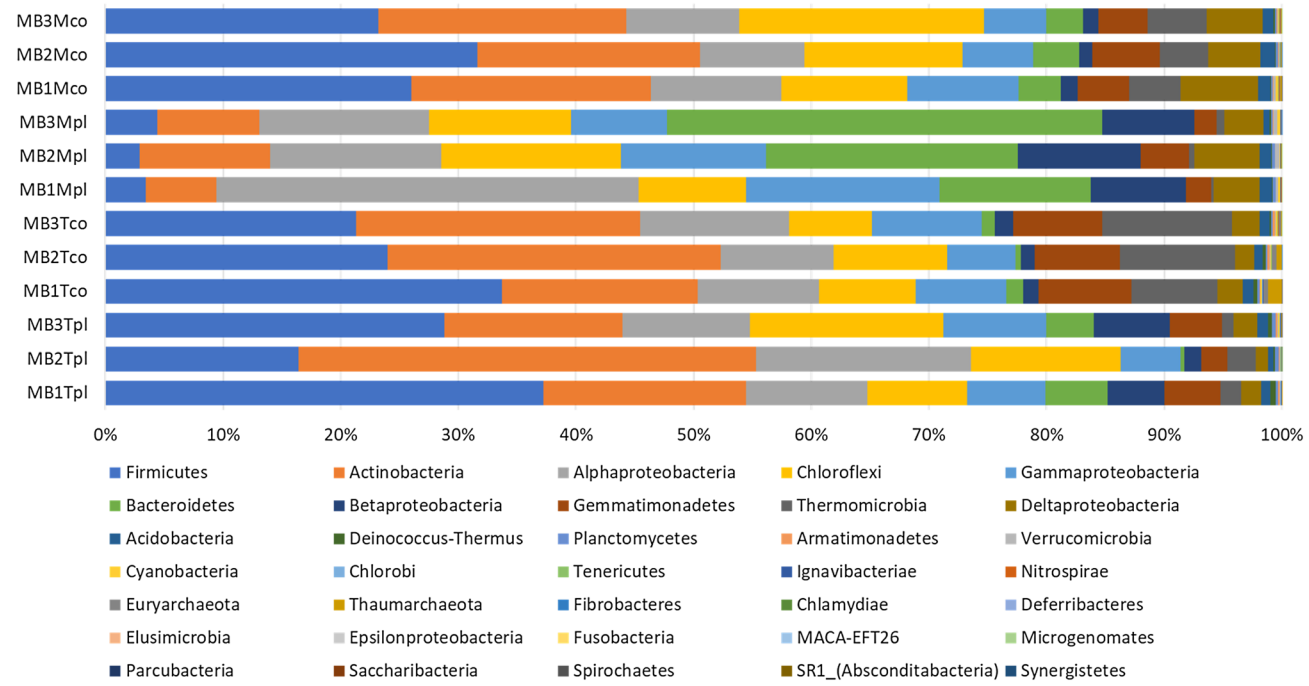

Fig. 4 Diversity of bacterial communities presented by a bar graph reporting the relative abundance in phylum in all Mater-Bi® samples

Sulfurifustis, *Ferrovibrio* and *Verticia* were identified associated to bioplastics (Figure S6). In addition, *Chryseolinea* and *Taibaiella* (both *Bacterodites*) were also two major genera detected, with, respectively, 7.06% and 4.01% associated to mesophilic plastics compared to 1.12% and 0.05% with thermophilic plastics.

Beside the previously described bacteria, some others appeared to be more specific to one or more bioplastics. In thermophilic conditions, *Firmicutes* were dominant in MB, in particular *Thermobacillus* (2.25%), *Paenibacillus* (1.94%) and *Cohnella* (4.01%). The *Aeribacillus* was also mostly associated to PBAT under thermophilic conditions (6.41%).

During the maturation phase, PBAT was colonized preferentially by *Verticia* (4%) and *Sulfurifustis* (2.88%), the latter one also present on MB (2.65%). The *Proteobacteria Limnobacter* (3.47%) and *Schlegelella* (7%), as well as *Actinobacteria Pseudomonas* (4.57%), and *Streptomyces* (3.28%) were more specific to PLA even if slightly present also in the other mesophilic plastics. Concerning LDPE, among *Proteobacteria* two thermophilic specialist bacteria were identified: *Cupriavidus* (6.13%) and *Rhizobium* (6.07%).

Enrichment cultures

Characterization

SEM observations did not report pronounced differences between samples incubated at 37 °C and at 58 °C (Fig. 6).

MB and PBAT had a thick layer of biofilm which ensured nutrients exchange between bioplastic surface and bacteria; microorganisms were distributed both on the surface and inside cracks of bioplastics (Fig. 6b–e). PLA and LDPE had less diffused and stratified biofilm, but more than before the enrichment cultures (Fig. 6f–g). From SEM micrographs, it was visible that the material structure of all the tested material was subjected to a strong degradation during the 2-month incubation, whatever the incubation conditions were: indeed, the remaining plastic residues did not display a homogeneous surface as at the start of incubation, but rather displayed cavities and cracks providing specific sites for bacterial settlement (particularly for PLA and PBAT). Lumps on LDPE surface were attributed to metabolites of microbial activity or to the tendency of the flat surface to flake off during degradation.

The FTIR spectra reported the presence of the major peaks which can be associated to the microbial communities development during the enrichment cultures: 1450 cm^{-1}, assigned to NH group of amides in the proteinaceous substances of biofilm [29], and 1635 cm^{-1}, related to carboxylate ions (R-COO-) formed due to microorganisms activity [30]. Interestingly, the main peaks of MB and PLA almost disappeared after incubation at 58 °C. For MB: 1710 cm^{-1} (C=O bounded to an alkyl group) and 1150–950 cm^{-1} (C–C in n-alkanes of starch). For PLA: 1748 cm^{-1} (C=O in lactide), 1132 cm^{-1} and 1081 cm^{-1} (C–C stretching in alkanes), 1181 cm^{-1} (C–O stretching) (Figure S7).

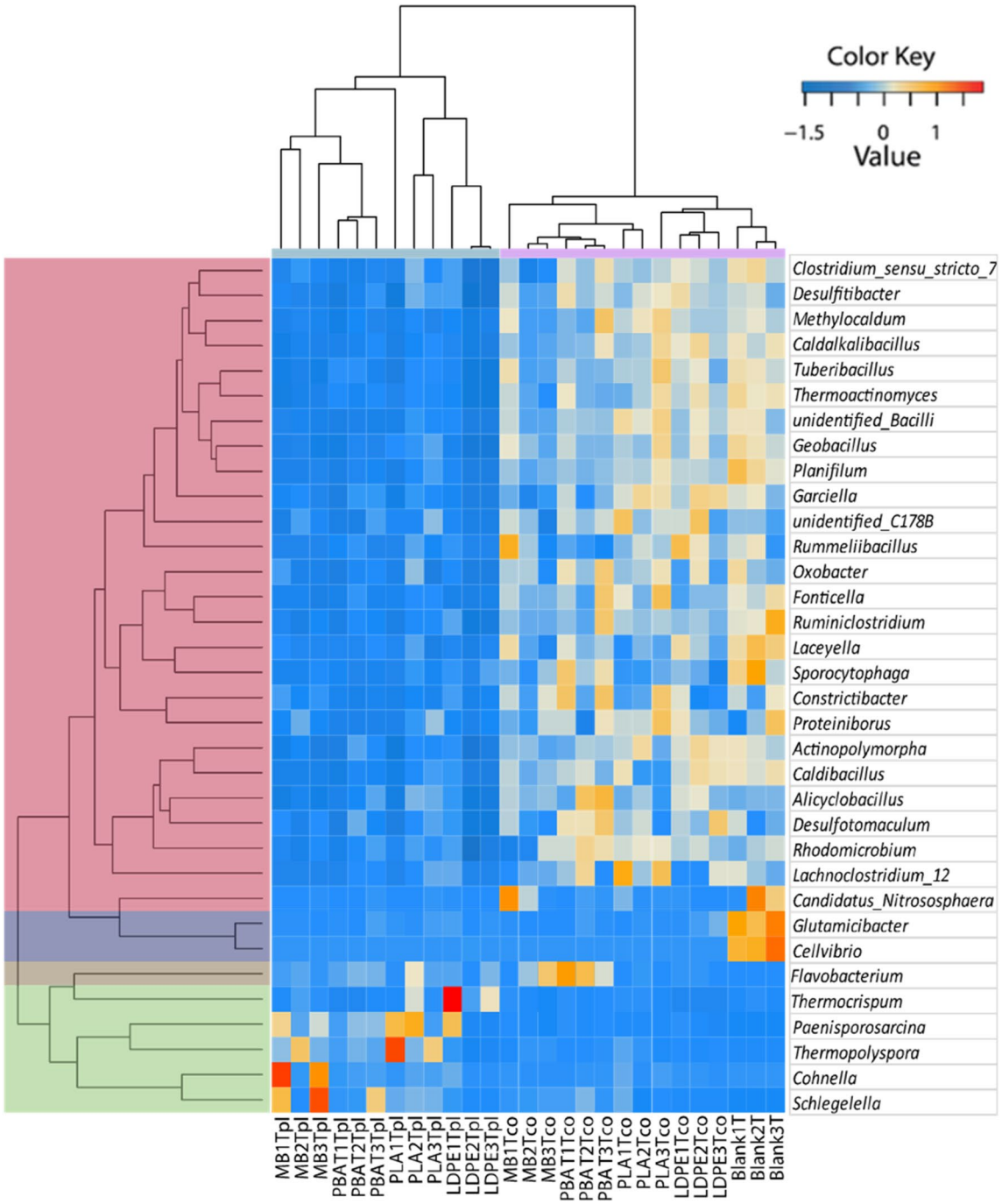

Fig. 5 Heat map of the relative abundance of the bacteria in genera. It compares all plastic types and compost under thermophilic conditions

Bacterial communities in the enrichment cultures

The 16S rRNA amplicon analysis of the 42 isolated bacteria was performed at the genus and species level. All the identified bacteria belong to the phylum of Firmicutes; they are reported in Table 2.

After incubation under thermophilic conditions, 13 isolates belonging to *Bacillus*, *Geobacillus and Parageobacillus* were identified. In particular, *Geobacillus thermodenitrificans* was found in PBAT and MB, one bacterial isolate associated with each polymer. *Geobacillus thermoleovorans* was isolated on PLA and LDPE, one isolate per plastic, and *Geobacillus kaustophilus* was also isolated on the same plastics but with, respectively, 1 and 4 isolates on PLA and LDPE. Two *Parageobacillus thermoglucosidasius* were identified, one in the culture of PBAT and one in PLA. *Bacillus borbori*

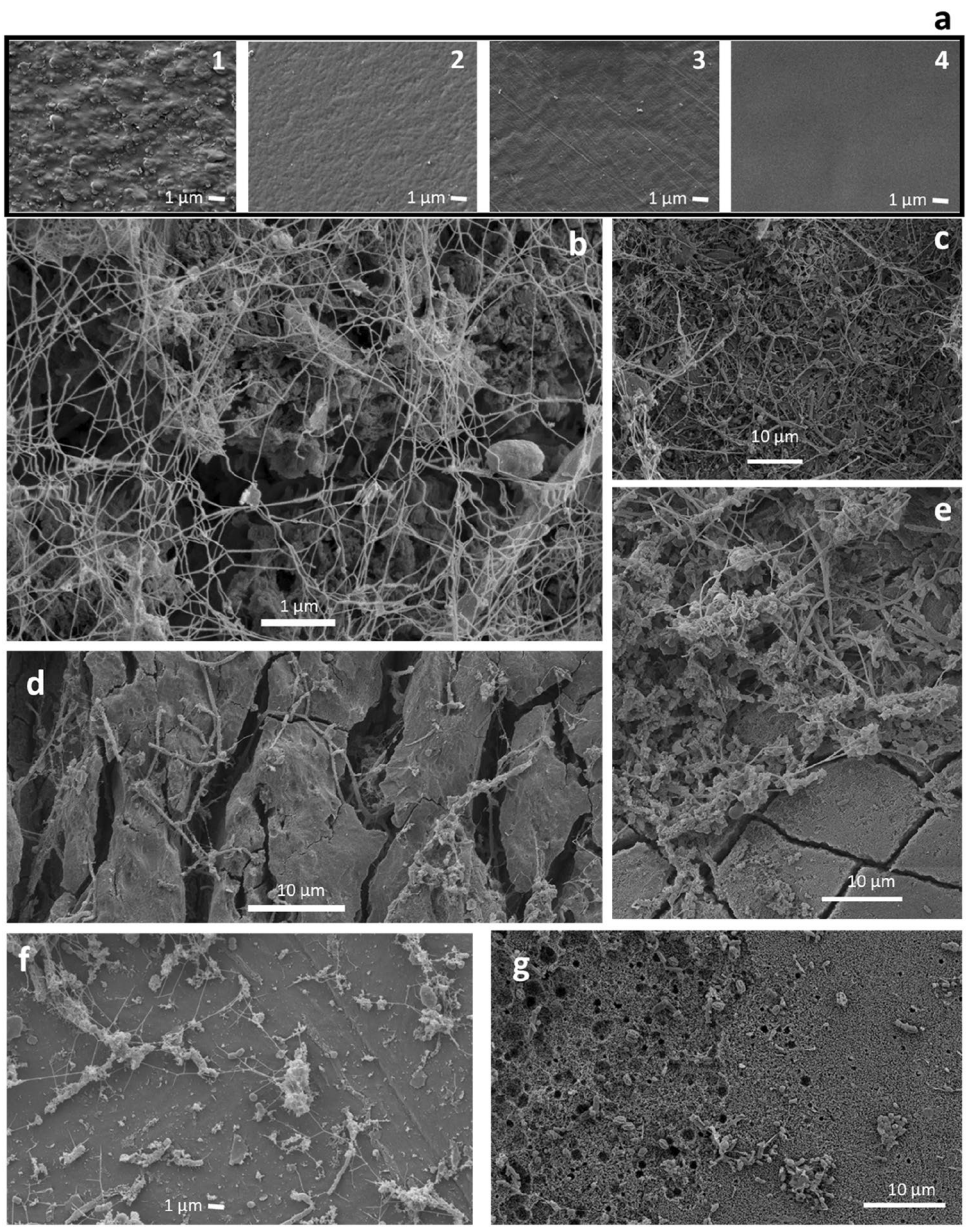

Fig. 6 SEM images of bioplastics surface after 2 months incubation at different temperature conditions: **a** bioplastics at the initial time 1. MB 2. PBAT 3. PLA 4. LDPE **b** MB at 58 °C; **c** MB at 37 °C; **d** PBAT at 58 °C, **e** PBAT at 37 °C, **f** LDPE at 37 °C and **g** PLA at 58 °C

Table 2 Bacteria isolated from enrichment cultures under thermophilic and mesophilic conditions

Genus	Species	Temperature conditions	Plastic
Bacillus	*borbori*	Thermophilic	MB
Bacillus	*cereus*	Mesophilic	MB, PBAT, LDPE
Bacillus	*cleronius*	Mesophilic	LDPE
Aeribacillus	*pallidus*	Thermophilic	PBAT, PLA, LDPE
Aneurinibacillus	*migulanus*	Mesophilic	MB, PBAT, PLA, LDPE
Brevibacillus	*borstelensis*	Thermophilic	MB
		Mesophilic	PBAT
Geobacillus	*thermoleovorans*	Thermophilic	PLA, LDPE
Geobacillus	*kaustophilus*	Thermophilic	PLA, LDPE
Parageobacillus	*thermoglucosidasius*	Thermophilic	PBAT, PLA

and *Brevibacillus borstelensis* were detected in MB culture. One *Brevibacillus borstelensis* was also isolated in PBAT under mesophilic conditions. Finally, *Aeribacillus pallidus* was detected with almost all the bioplastics: 3, 4 and 1 bacterial species were isolated, respectively, in PBAT, PLA and LDPE.

After incubation under mesophilic conditions, 20 isolates were obtained belonging to four different species, and therefore, a smaller number compared to the thermophilic conditions. Most of them were present in almost all the plastics and, therefore, was not specific to one material. Beside the already mentioned *Brevibacillus borstelensis* isolated in PBAT, one *Bacillus oleronius* was found in the isolation process with LDPE. Then, *Bacillus cereus* was identified with almost all the bioplastics. 3, 2 and 2 bacteria of this species were isolated, respectively, in MB, PBAT and LDPE. Finally, *Aneurinibacillus migulanus* was identified with all the bioplastics: 2 were found in MB, 2 in PBAT, 5 in PLA and 2 in LDPE.

Discussion

Bacterial community structure

In the preliminary part of the test, fully described in Ruggero et al. 2021, it was found that while bioplastics presented degradation signs at the end of composting tests, conventional LDPE was still undegraded and did not present a wide bacterial colonization [23].

Despite these differences, the amplicon sequencing disclosed that all the tested materials were increasingly colonized by bacteria. Moreover, the highest relative abundance of specialized bacteria was detected in LDPE; unexpectedly, this conventional plastic displayed the lowest degradation. It is fair to assume that the less biodegradable is a compound, the more specialized is the group of bacteria exploiting the compound as carbon source. Indeed, bacteria that may have the capability to produce enzymes useful to degrade the complex structure of the polymer can predominate, as observed from the equitability index (Fig. 2b). On the contrary, a polymer with more readily biodegradable compounds, such as MB and PLA, can be colonized by a wider group of bacteria without specific metabolism. In the present study, a large variety of bacteria was identified in compost matrix, which have already been detected in previous research carried out in composting environment: e.g., *Geobacillus* [31], *Tuberibacillus* [32], *Planifilum* [33], and *Hydrogenispora*. In line with the literature, the present research outlined a significant variation of bacteria according to the composting conditions and plastic types [34, 35], as shown by nMDS profile (Fig. 1). In particular, temperature had an impact on the structure of the bacterial community [36], and bacteria in the thermophilic phase needed to face high temperature conditions by, for example, forming heat-resistance endospores, as commonly done by most of Firmicutes [37] and by *Thermopolyspora* [38]. Moreover, the bacterial community originally present in the harvested compost changed significantly during the degradation process, and the abundance of polymers degrading bacteria increased from day 0, to day 20 and finally to day 60 [39], with the progressive appearance of, e.g., *Paenibacillus*, *Pseudomonas*, *Schlegellela*, *Sulfurifustis*, and *Pigmetiphaga* (Figure S6). This latter one was found specifically in wastewater containing dying agents, for the capacity to use pigments of the plastics [40]. However, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes* and *Chloroflexi* always remained the most represented phyla in the bacterial community of both compost and plastics. The same predominance was already assessed in previous studies carried out in compost both during the thermophilic and the maturation phase [41–43].

These findings led to the following consideration: a core bacterial consortium is maintained in the compost, while specific plastic degraders progressively appeared during the 60-day composting process, as confirmed by the Venn diagrams. This also means that the concentration of bioplastics in compost (2.9%), as well as the duration of the composting phases, was enough to change the bacterial community towards a more specialized in degrading polymers.

Focusing on specific genera associated to plastics degradation in this study, a comparison with the literature was carried out (Table 3). It was found that some of the specialized bacteria identified in Table 3 may be capable to produce enzymes typically involved in hemicellulose, chitin and lignin degradation in composting. Chitinase enzymes degrade the chitin, a fibrous substance consisting of polysaccharides. *Cohnella* was associated in previous studies to the production of chitinases [44, 45]. Like chitin, hemicellulose and cellulose are slower to be degraded in compost than sugars, starch, lipids, acids and proteins, but faster than lignin. These compounds are degraded by xylanase enzymes [46]. Regarding the bacteria associated in this study to bioplastics degradation, *Thermopolyspora* was found to be able to produce xylanase enzymes in a previous research [47]. Finally, concerning lignin, scission reactions by specific enzymes are more difficult. The enzymes capable to degrade lignin can interact with low molecular weight that could lead to the formation of free radicals and consequently to oxidize and to cleave complex macromolecular lignin network [3, 48]. The oxidizing ability of such microorganisms can also contribute to the degradation mechanisms of complex molecular structure of polymers. Previous research about polymeric blends degradation, in particular polyethylene and starch-based, reported evident signs of erosion in the presence of

Table 3 Comparison between the microbial genera highlighted by 16S rRNA amplicon sequencing (data from the heatmaps) and found in the literature

This study			In the literature	
Genus detected by amplicon sequencing	Temperature condition	Tested material	Plastic with the genus	Ref.
Thermopolyspora	Thermophilic	PLA		*
Streptomyces	Mesophilic	PLA	PHA; PHB polyester	[53, 73, 74]
Bacillus, *Geobacillus*,	Thermophilic	All	PHA; PHB PBAT	[39, 53, 73]
Paenibacillus	Thermophilic	MB	PBAT	[75, 76]
Cohnella	Thermophilic	MB		*
Aeribacillus	Thermophilic	PBAT		*
Pigmentiphaga, *Sulfurifustis*	Mesophilic	MB, PBAT		*
Ferrovibrio, *Verticia*	Mesophilic	PBAT		*
Pseudomonas	Mesophilic	PLA	PLA; PHA	[53, 77]
Schlegellela, *Limnobacter*	Mesophilic	PLA	PHB	[43]
Cupriavidus	Thermophilic	LDPE	PHA	[37]
Rhizobium	Mesophilic	LDPE, PBAT	PET	[21]

*No reference about these genera associated to plastic degradation

lignin-degrading bacteria of the genus Streptomyces [43, 49]. *Streptomyces* was also identified in this study among PLA-degrading genera.

Some other bacteria were found to be abundant in the community structure (Figure S5 and S6). The following bacterial species are not mentioned in Table 3, as they were not directly associated to plastics degradation: *Steroidobacter*, *Sphaerobacter*, *Chryseolinea*, *Taibaiella* and *Iamia*. Though, they can be considered as a part of the core bacterial consortium shared among the tested samples. Most of them are known from previous research for their abundance in compost and soil [50, 51]. These bacteria cannot produce any of the previously described enzymes involved in polymers degradation mechanisms. However, that e.g., *Taibaiella* and *Iamia* have a metabolism capable to easily degrade some shorter acids and sugars which can be intermediate products of plastics degradation [42, 51]. Thus, the tested materials can indirectly be an additional suitable substrate in compost. It is fair to report that some environmental factors may also have affected these bacteria survive in composting. In particular, in previous studies, it was assessed that temperature, pH, moisture content, and NH_4^+ concentration were the primary environmental variables influencing microbial genus composition, and each genus was affected to a different degree by these abiotic factors [42, 52].

Finally, important references were found in the literature about some plastic-degrading bacteria, which have not yet been mentioned in the present study. However, an oriented search of these species in the heatmaps detected some of these bacteria in the community. Their low abundance prevented the association with bioplastics degradation at first screening. In particular, *Brevibacillus* and *Clostridium* in the phylum of *Firmicutes* were associated in a previous study to PLA and PBAT deterioration [53, 54]. In the phylum of *Proteobacteria*, *Alcanivorax* was found to play a fundamental role in the degradation of LDPE [28], and finally in the phylum of *Actinobacteria*, *Saccharopolyspora* was identified in a previous study for its abundance in soil reached in microplastics [55].

Enrichment cultures

It was disclosed that Firmicutes dominated both the thermophilic and the mesophilic communities in the enrichment cultures. This phylum was commonly recognized as a fermenting group of bacteria [37], better heat-resistant than other phyla [56]. Considering that LB is generally suitable medium for various enrichment cultures [27], it is assumable that the predominance of Firmicutes was mostly relatable to their much better adaptability to the cultivation conditions. Moreover, *Bacillus* and *Geobacillus*, highly developed in the cultures, were abundant in bioplastic samples already before cultivation. Interestingly, the SEM images taken after the enrichment process (Fig. 6) already provided a first indication of dominant microorganism grown on bioplastic surface. Indeed, comparing our SEM images with other papers, the microorganisms grown may be associated with genera *Geobacillus*, *Bacillus* and *Brevibacillus* [57–59], which were then isolated in this study with amplicon sequencing (Table 2).

From a comparison with previous studies in the literature, *Geobacillus thermodenitrificans* was found to be capable to use starch for its metabolism, in particular the strain *BGSC* [60]. Indeed, in the current work, this species was identified in the cultivation with MB bioplastic containing starch. It is also interesting to report that *Geobacilli* are generally

capable to exploit n-alkanes present in the polymers as substrate, i.e., the species *thermoleovorans* and *kaustophilus* [61], and *thermoglucosidasius* of *Parageobacilli*, the strain *ATCC 43742* [62].

Among the colonizing *Firmicutes*, *Aeribacillus pallidus* was detected in almost all the bioplastics in thermophilic conditions, while before cultivation it was predominant only in MB. Moreover, *Brevibacillus*, one of the plastic-degrading bacteria with low abundance in the original community, colonized MB and PBAT in both mesophilic and thermophilic conditions. This finding confirmed what disclosed by previous authors about the capability of *Brevibacillus* to exploit PBAT as sole carbon source [53]. PBAT is also the prevalent component of MB polymeric structure. Both *Brevibacillus borstelensis* and *Aeribacillus pallidus* were furtherly investigated in the literature, finding that specific strains can be capable to degrade complex carbonic structure, respectively, the strain *DX-4* [63] and the strain *C10* [64]. Concerning *Brevibacillus borstelensis*, the strain *707* was studied by Hadad et al., 2005 as specialist in LDPE degradation in soil [65]; however, in the LDPE cultures of the present study, *Brevibacillus borstelensis* was not identified.

Conversely, from the isolation process with LDPE, it was found *Bacillus oleronius*: it has the capacity to adapt to mesophilic conditions, but is also known for its ability to cause extended lesions in skin and blepharitis [66] [67]. *Bacillus cereus* was isolated from LDPE, but also from MB and PBAT. It was found to well adapt to mesophilic conditions; moreover, this species present many strains with different performances and capabilities [68]. *Bacillus cereus* can be found in soil, water and food, where it is a potential pathogen for humans [69]. In particular, the strain *ATCC 14579* exploits a large number of carbon sources, such as starch and other saccharides, amino acids, dipeptides and glycerol, while other strains of the same bacillus are not able to use these nutrients for their growth [70]. Finally, *Aneurinibacillus migulanus* was slightly present in the community before the culture, but at the end of the test, it was isolated in all the mesophilic cultures. This bacterium cannot produce any specific enzyme involved in polymers degradation and it is generally used against plants diseases for its antibiotic effect [71, 72]. Therefore, it is assumable that *Aneurinibacillus migulanus* did not survive in the enrichment cultures for its capability to use polymers as carbon source, but due to an endogenous growth.

Conclusions

A composting test was carried out in the present study, simulating operating conditions of industrial aerobic organic waste treatments. After 2-month composting, the tested bioplastics showed deep signs of erosion, in particular Mater-Bi®. On the contrary, conventional LDPE was not subjective to a significative degradation. Regarding the aim to determine if bioplastics influence the microbial diversity of compost, the present study disclosed that 16S rRNA profile in bioplastics-associated communities largely differed from that of the initial compost (positive control), harvested to carry out the test. In addition, thanks to the 16S rRNA amplicon sequencing, the current research identified some bacteria which have a higher abundance in one of the tested materials with respect to the others. Some specialists were found to be plastic nature dependent, but a larger variety of bacteria colonized two or more plastic types. This finding is important in the promotion of research studies on bioaugmentation. Indeed, bioaugmentation practice can enhance the degradation of different kinds of bioplastic in industrial composting plants (LDPE is not compostable).

A further important finding of the study was that bacterial communities developing on bioplastics and LDPE hosted distinct bacteria in mesophilic and thermophilic conditions. As expected, during the thermophilic phase there was a prevalence of better heat-resistant phyla compared to the mesophilic phase.

Finally, with the objective to select bacterial species capable of growing on the tested bioplastics, applying an enrichment method, it was disclosed that *Firmicutes* completely predominated in bioplastics colonization, under both thermophilic and mesophilic conditions. Again, most of the species identified with the enrichment approach was found to be specialist of two or more tested plastics.

However, considering the results of the amplicon sequencing, some genera capable to specifically degrade bioplastics were identified also among *Proteobacteria* and *Actinobacteria*. Therefore, enrichment methods—enhancing the growth of species from these phyla—may be further investigated.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Calabrò PS, Grosso M (2018) Bioplastics and waste management. Waste Manag 78:800–801. https://doi.org/10.1016/j.wasman.2018.06.054

2. Consorzio Italiano Compostatori (2017) Dati di settore, rapporto CIC 2017. 69–71
3. Lucas N, Bienaime C, Belloy C et al (2008) Polymer biodegradation: mechanisms and estimation techniques—a review. Chemosphere 73:429–442. https://doi.org/10.1016/j.chemosphere.2008.06.064
4. Shah AA, Hasan F, Hameed A, Ahmed S (2008) Biological degradation of plastics: a comprehensive review. Biotechnol Adv 26:246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005
5. Kumaravel S, Hema R, Lakshmi R (2010) Production of polyhydroxybutyrate (Bioplastic) and its biodegradation by pseudomonas lemoignei and *Aspergillus niger*. E J Chem 7:1–4. https://doi.org/10.1155/2010/148547
6. Tokiwa Y, Calabia BP (2004) Degradation of microbial polyesters. Biotechnol Lett 26:1181–1189. https://doi.org/10.1023/B:BILE.0000036599.15302.e5
7. Folino A, Karageorgiou A, Calabrò PS, Komilis D (2020) Biodegradation of wasted bioplastics in natural and industrial environments: a review. Sustainability. https://doi.org/10.3390/su12156030
8. Emadian SM, Onay TT, Demirel B (2017) Biodegradation of bioplastics in natural environments. Waste Manag 59:526–536. https://doi.org/10.1016/j.wasman.2016.10.006
9. Dey S, Rout AK, Behera BK, Ghosh K (2022) Plastisphere community assemblage of aquatic environment: plastic-microbe interaction, role in degradation and characterization technologies. Environ Microbiomes 17:1–21. https://doi.org/10.1186/s40793-022-00430-4
10. Zettler ER, Mincer TJ, Amaral-Zettler LA (2013) Life in the "plastisphere": microbial communities on plastic marine debris. Environ Sci Technol 47:7137–7146. https://doi.org/10.1021/es401288x
11. Bandini F, Misci C, Taskin E et al (2020) Biopolymers modulate microbial communities in municipal organic waste digestion. FEMS Microbiol Ecol 96:1–13. https://doi.org/10.1093/femsec/fiaa183
12. Gu JD, Mitchell R, Ford TE (2003) Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. Int Biodeterior Biodegrad 52:69–91. https://doi.org/10.1016/S0964-8305(02)00177-4
13. Rocha-Santos TAP, Duarte AC (2017) Characterization and analysis of microplastics, vol 75, (**ISBN 9780444638984**)
14. Ruggero F, Gori R, Lubello C (2019) Methodologies to assess biodegradation of bioplastics during aerobic composting and anaerobic digestion: a review. Waste Manag Res. https://doi.org/10.1177/0734242X19854127
15. Massardier-Nageotte V, Pestre C, Cruard-Pradet T, Bayard R (2006) Aerobic and anaerobic biodegradability of polymer films and physico-chemical characterization. Polym Degrad Stab 91:620–627. https://doi.org/10.1016/j.polymdegradstab.2005.02.029
16. EN 13432 (2000) Packaging—Requirements for packaging recoverable through composting and biodegradation—Test scheme and evaluation criteria for the final acceptance of packaging
17. ISO 20200 (2015) Test, Standards Publication Plastics—Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale
18. ASTM D5338 (2011) Standard test method for determining aerobic biodegradation of plastic materials under controlled composting conditions, incorporating thermophilic temperatures. ASTM International
19. European Commission (2017) Report from the commission to the european parliament, the council, the european economic and social committee and the committee of the regions. Off J Eur Union COM (2017):1–14
20. Bastioli C (1998) Properties and applications of Mater-Bi starch-based materials. Polym Degrad Stab 59:263–272
21. ISO 14855-2 (2018) Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions - Method by analysis of evolved carbon dioxide
22. ISO 11465 (1993) Soil quality—Determination of dry matter and water content on a mass basis—Gravimetric method
23. Ruggero F, Onderwater RCA, Carretti E, et al (2021) Degradation of film and rigid bioplastics during the thermophilic phase and the maturation phase of simulated composting. J Polym Environ 29:3015–3028
24. Barbier FF, Chabikwa TG, Ahsan MU et al (2019) A phenol/chloroform-free method to extract nucleic acids from recalcitrant, woody tropical species for gene expression and sequencing. Plant Methods 15:9–12. https://doi.org/10.1186/s13007-019-0447-3
25. Wang Y, Naumann U, Wright ST, Warton DI (2012) Mvabund- an R package for model-based analysis of multivariate abundance data. Methods Ecol Evol 3:471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x
26. Dixon P (2003) Computer program review VEGAN, a package of R functions for community ecology. J Veg Sci 14:927–930
27. Yoshida S, Hiraga K, Takehana T et al (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science (80-). https://doi.org/10.1126/science.aaf8305
28. Delacuvellerie A, Cyriaque V, Gobert S et al (2019) The plastisphere in marine ecosystem hosts potential specific microbial degraders including Alcanivorax borkumensis as a key player for the low-density polyethylene degradation. J Hazard Mater 380:120899. https://doi.org/10.1016/j.jhazmat.2019.120899
29. Bonhomme S, Cuer A, Delort AM et al (2003) Environmental biodegradation of polyethylene. Polym Degrad Stab 81:441–452. https://doi.org/10.1016/S0141-3910(03)00129-0
30. Arrieta MP, López J, Rayón E, Jiménez A (2014) Disintegrability under composting conditions of plasticized PLA–PHB blends. Polym Degrad Stab 108:307–318. https://doi.org/10.1016/j.polymdegradstab.2014.01.034
31. Poli A, Laezza G, Gul-Guven R et al (2011) *Geobacillus galactosidasius* sp. nov., a new thermophilic galactosidase-producing bacterium isolated from compost. Syst Appl Microbiol 34:419–423. https://doi.org/10.1016/j.syapm.2011.03.009
32. Hatayama K, Shoun H, Ueda Y, Nakamura A (2006) *Tuberibacillus calidus* gen. nov., sp. nov., isolated from a compost pile and reclassification of *Bacillus naganoensis* Tomimura et al. 1990 as *Pullulanibacillus naganoensis* gen. nov., comb. Nov. and *Bacillus laevolacticus* Andersch et al. 1994 as Sporolacto. Int J Syst Evol Microbiol 56:2545–2551. https://doi.org/10.1099/ijs.0.64303-0
33. Han SI, Lee JC, Lee HJ, Whang KS (2013) *Planifilum composti* sp. nov., a thermophile isolated from compost. Int J Syst Evol Microbiol 63:4557–4561. https://doi.org/10.1099/ijs.0.053199-0
34. Liu H, Huang Y, Duan W et al (2020) Microbial community composition turnover and function in the mesophilic phase predetermine chicken manure composting efficiency. Bioresour Technol 313:123658. https://doi.org/10.1016/j.biortech.2020.123658
35. Wang X, Pan S, Zhang Z et al (2017) Effects of the feeding ratio of food waste on fed-batch aerobic composting and its microbial community. Bioresour Technol 224:397–404. https://doi.org/10.1016/j.biortech.2016.11.076
36. Chen Z, Zhang S, Wen Q, Zheng J (2015) Effect of aeration rate on composting of penicillin mycelial dreg. J Environ Sci 37:172–178
37. Sangeetha T, Guo Z, Liu W et al (2017) Energy recovery evaluation in an up flow microbial electrolysis coupled anaerobic digestion (ME-AD) reactor: Role of electrode positions and hydraulic retention times. Appl Energy 206:1214–1224. https://doi.org/10.1016/j.apenergy.2017.10.026

38. Goodfellow M, Maldonado LA, Quintana ET (2005) Reclassification of *Nonomuraea flexuosa* (Meyer 1989) Zhang et al. 1998 as *Thermopolyspora flexuosa* gen. nov., comb. nov., nom. rev. Int J Syst Evol Microbiol 55:1979–1983. https://doi.org/10.1099/ijs.0.63559-0
39. Zhang M, Jia H, Weng Y, Li C (2019) Biodegradable PLA/PBAT mulch on microbial community structure in different soils. Int Biodeterior Biodegrad 145:104817. https://doi.org/10.1016/j.ibiod.2019.104817
40. Yoon JH, Kang SJ, Kim W, Oh TK (2007) Pigmentiphaga daeguensis sp. nov., isolated from wastewater of a dye works, and emended description of the genus Pigmentiphaga. Int J Syst Evol Microbiol 57:1188–1191. https://doi.org/10.1099/ijs.0.64901-0
41. Cerda A, Artola A, Font X et al (2018) Composting of food wastes: status and challenges. Bioresour Technol 248:57–67
42. Zhong XZ, Ma SC, Wang SP et al (2018) A comparative study of composting the solid fraction of dairy manure with or without bulking material: performance and microbial community dynamics. Bioresour Technol 247:443–452. https://doi.org/10.1016/j.biortech.2017.09.116
43. Kong W, Sun B, Zhang J et al (2020) Metagenomic analysis revealed the succession of microbiota and metabolic function in corncob composting for preparation of cultivation medium for *Pleurotus ostreatus*. Bioresour Technol 306:123156. https://doi.org/10.1016/j.biortech.2020.123156
44. Aliabadi N, Aminzadeh S, Karkhane AA, Haghbeen K (2016) Thermostable chitinase from *Cohnella* sp. A01: isolation and product optimization. Brazilian J Microbiol 47:931–940. https://doi.org/10.1016/j.bjm.2016.07.009
45. Narancic T, Cerrone F, Beagan N, O'Connor KE (2020) Recent advances in bioplastics: application and biodegradation. Polymers (Basel). https://doi.org/10.3390/POLYM12040920
46. Quitadamo A, Massardier V, Iovine V et al (2019) Effect of cellulosicwaste derived filler on the biodegradation and thermal properties of HDPE and PLA composites. Processes. https://doi.org/10.3390/pr7100647
47. Anbarasan S, Wahlström R, Hummel M et al (2017) High stability and low competitive inhibition of thermophilic *Thermopolyspora flexuosa* GH10 xylanase in biomass-dissolving ionic liquids. Appl Microbiol Biotechnol 101:1487–1498. https://doi.org/10.1007/s00253-016-7922-9
48. Peelman N, Ragaert P, De Meulenaer B et al (2013) Application of bioplastics for food packaging. Trends Food Sci Technol 32:128–141. https://doi.org/10.1016/j.tifs.2013.06.003
49. Lee B, Pometto AL, Fratzke A, Bailey TB (1991) Biodegradation of degradable plastic polyethylene by Phanerochaete and *Streptomyces* species. Appl Environ Microbiol 57:678–685. https://doi.org/10.1128/aem.57.3.678-685.1991
50. Storey S, Chualain DN, Doyle O et al (2015) Comparison of bacterial succession in green waste composts amended with inorganic fertiliser and wastewater treatment plant sludge. Bioresour Technol 179:71–77. https://doi.org/10.1016/j.biortech.2014.11.107
51. Xu L, Yi M, Yi H et al (2018) Manure and mineral fertilization change enzyme activity and bacterial community in millet rhizosphere soils. World J Microbiol Biotechnol. https://doi.org/10.1007/s11274-017-2394-3
52. Maeda K, Hanajima D, Morioka R, Osada T (2010) Characterization and spatial distribution of bacterial communities within passively aerated cattle manure composting piles. Bioresour Technol 101:9631–9637. https://doi.org/10.1016/j.biortech.2010.07.057
53. Boyandin AN, Prudnikova SV, Karpov VA et al (2013) Microbial degradation of polyhydroxyalkanoates in tropical soils. Int Biodeterior Biodegrad 83:77–84. https://doi.org/10.1016/j.ibiod.2013.04.014
54. Ghosh SK, Pal S, Ray S (2013) Study of microbes having potentiality for biodegradation of plastics. Environ Sci Pollut Res Int 20:4339–4355. https://doi.org/10.1007/s11356-013-1706-x
55. Nakei MD (2015) Isolation and identification of plastics-degrading microorganisms from soils of Morogoro, Tanzania
56. Zhang H, McGill E, Gomez CO et al (2017) Disintegration of compostable foodware and packaging and its effect on microbial activity and community composition in municipal composting. Int Biodeterior Biodegrad 125:157–165. https://doi.org/10.1016/j.ibiod.2017.09.011
57. Ramarao N, Tran SL, Marin M, Vidic J (2020) Advanced methods for detection of Bacillus cereus and its pathogenic factors. Sensors (Switzerland). https://doi.org/10.3390/s20092667
58. Khalil AB, Sivakumar N, Arslan M et al (2018) Insights into *Brevibacillus borstelensis* AK1 through whole genome sequencing: a thermophilic bacterium isolated from a hot spring in Saudi Arabia. Biomed Res Int. https://doi.org/10.1155/2018/5862437
59. Hermabessiere L, Himber C, Boricaud B et al (2018) Optimization, performance, and application of a pyrolysis-GC/MS method for the identification of microplastics. Anal Bioanal Chem 410(25):6663–6676. https://doi.org/10.1007/s00216-018-1279-0
60. Manachini PL, Mora D, Nicastro G et al (2000) *Bacillus thermodenitrificans* sp. nov., nom. rev. Int J Syst Evol Microbiol 50:1331–1337. https://doi.org/10.1099/00207713-50-3-1331
61. Nazina TN, Tourova TP, Poltaraus AB et al (2001) Taxonomic study of aerobic thermophilic bacilli: Descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus th*. Int J Syst Evol Microbiol 51:433–446
62. Inoue M, Tanimura A, Ogami Y, et al (2019) Draft Genome Sequence of *Parageobacillus thermoglucosidasius*. Microbiol Resour Announc 8(5). https://doi.org/10.1128/mra.01666-18
63. Wang YQ, Yuan Y, Yu Z et al (2013) *Bacillus borbori* sp. Nov., Isolated from an electrochemically active biofilm. Curr Microbiol 67:718–724. https://doi.org/10.1007/s00284-013-0426-2
64. Yildirim V, Baltaci MO, Ozgencli I et al (2017) Purification and biochemical characterization of a novel thermostable serine alkaline protease from *Aeribacillus pallidus* C10: a potential additive for detergents. J Enzyme Inhib Med Chem 32:468–477. https://doi.org/10.1080/14756366.2016.1261131
65. Hadad D, Geresh S, Sivan A (2005) Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. J Appl Microbiol 98:1093–1100. https://doi.org/10.1111/j.1365-2672.2005.02553.x
66. Owusu-Darko R, Allam M, Mtshali S et al (2017) Draft genome sequence of *Bacillus oleronius* DSM 9356 isolated from the termite *Reticulitermes santonensis*. Genom Data 12:76–78. https://doi.org/10.1016/j.gdata.2017.03.005
67. Szkaradkiewicz A, Chudzicka-Strugala I, Karpiński TM et al (2012) *Bacillus oleronius* and Demodex mite infestation in patients with chronic blepharitis. Clin Microbiol Infect 18:1020–1025. https://doi.org/10.1111/j.1469-0691.2011.03704.x
68. Sen R, Tripathy S, Padhi SK et al (2015) Assessment of genetic diversity of *Bacillus* spp. isolated from eutrophic fish culture pond. 3 Biotech 5:393–400. https://doi.org/10.1007/s13205-014-0234-9
69. Ivanova N, Sorokin A, Anderson I et al (2003) Genome sequence of *Bacillus cereus* and comparative analysis with *Bacillus anthracis*. Nature 423:87–91. https://doi.org/10.1038/nature01582
70. Mols M, De Been M, Zwietering MH et al (2007) Metabolic capacity of *Bacillus cereus* strains ATCC 14579 and ATCC 10987 interlinked with comparative genomics. Environ Microbiol 9:2933–2944. https://doi.org/10.1111/j.1462-2920.2007.01404.x
71. Alenezi FN, Rekik I, Bouket AC et al (2017) Increased biological activity of *Aneurinibacillus migulanus* strains correlates with

the production of new gramicidin secondary metabolites. Front Microbiol 8:1–11. https://doi.org/10.3389/fmicb.2017.00517
72. Takagi H, Shida O, Kadowaki K et al (1993) Characterization of *Bacillus brevis* with descriptions of *Bacillus migulanus* sp. nov., *Bacillus choshinensis* sp. nov., *Bacillus parabrevis* sp. nov., and *Bacillus galactophilus* sp. nov. Int J Syst Bacteriol 43:221–231. https://doi.org/10.1099/00207713-43-2-221
73. Hsu KJ, Tseng M, Don TM, Yang MK (2012) Biodegradation of poly(β-hydroxybutyrate) by a novel isolate of streptomyces bangladeshensis 77T-4. Bot Stud 53:307–313
74. Trinh Tan F, Cooper DG, Marić M, Nicell JA (2008) Biodegradation of a synthetic co-polyester by aerobic mesophilic microorganisms. Polym Degrad Stab 93:1479–1485. https://doi.org/10.1016/j.polymdegradstab.2008.05.005
75. Jeszeová L, Puškárová A, Bučková M, Kraková L, Grivalský T, Danko M, Mosnáčková K, Chmela Š, Pangallo D (2018) Microbial communities responsible for the degradation of poly(lactic acid)/poly(3-hydroxybutyrate) blend mulches in soil burial respirometric tests. World J Microbiol Biotechnol 34:101. https://doi.org/10.1007/s11274-018-2483-y
76. Teeraphatpornchai T, Nakajima-Kambe T, Shigeno-Akutsu Y, Nakayama M, Nomura NTN, Uchiyama H (2003) Isolation and characterization of a bacterium that degrades PBSA. Biotechnol Lett 25:23–28
77. Pattanasuttichonlakul W, Sombatsompop N, Prapagdee B (2018) Accelerating biodegradation of PLA using microbial consortium from dairy wastewater sludge combined with PLA-degrading bacterium. Int Biodeterior Biodegrad 132:74–83. https://doi.org/10.1016/j.ibiod.2018.05.014