Variation in the bacterial and fungal communities over a three-year period at Mexico City's largest operational-capacity composting plant

Variación en las comunidades bacterianas y fúngicas durante un período de tres años en la planta de compostaje con mayor capacidad operativa de la Ciudad de México

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Abstract

In Mexico City, a daily generation of 12404 t of municipal solid waste was registered in 2022. Approximately 1374 t of organic matter are received at the Bordo Poniente Composting Plant (BPCP, West Rim Composting Plant) for transformation into compost. BPCP is likely the largest composting facility in Latin America. A total of 15 samples were collected over a three-year period after its opening in 2012. The samples were then subjected to analysis to ascertain their moisture content, pH, reducing sugar content, C/N ratio, and maximum CO₂ production rate. All the analyzed samples were found to meet the requisite criteria for stable compost. The polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method was employed to identify the bacterial and fungal communities present in the samples. The descriptive analysis revealed the presence of seven bacteria and three fungi in all the compost samples coming from the rainy and dry seasons; thereby generating basic knowledge of the microbiota composition suitable for the composting process. Additionally, some of the identified microorganisms have biotechnological potential for application in different industrial sectors. The data obtained are highly relevant and remain so despite the time elapsed since the sampling. They shed light on the microbial dynamics during the composting process, with a broad approach (bacteria and fungi). Most of previous studies have only focused on one of these taxonomic categories, which misses a lot of information on the complex syntrophic relationships that may occur. The complexity and size of the composting plant studied is another element of great interest.

Keywords: Bacterial and fungal communities, compost, compost stability, DGGE-PCR, nested PCR.

Resumen

En 2022 se generaron diariamente 12404 t de residuos sólidos urbanos en la Ciudad de México. Aproximadamente 1374 t de materia orgánica se reciben en la Planta de Composta Bordo Poniente (BPCP) para su transformación en composta, siendo esta planta, muy probablemente, la mayor instalación de compostaje de toda América Latina. Un total de 15 muestras fueron recogidas durante un período de tres años después de su apertura en 2012. Las muestras se sometieron a análisis para determinar su contenido de humedad, pH, contenido de azúcares reductores, relación C/N y tasa máxima de producción de CO₂. Se comprobó que todas las muestras analizadas cumplían los criterios exigidos para una composta estable. Para identificar las comunidades bacterianas y fúngicas presentes en las muestras se empleó el método de reacción en cadena de la polimerasa-y electroforesis en gel de gradiente desnaturalizante (PCR-DGGE). El análisis descriptivo reveló la presencia de siete bacterias y tres hongos en todas las muestras de composta provenientes de las estaciones de lluvia y seca, generando así un conocimiento básico de la composición de la microbiota adecuada para el proceso de compostaje. Además, algunos de los microorganismos identificados tienen potencial biotecnológico para su aplicación en diferentes sectores industriales. Los datos obtenidos son altamente relevantes y siguen siéndolo a pesar del tiempo transcurrido desde el muestreo. Permiten comprende mejor la dinámica microbiana durante el proceso de compostaje, con un enfoque amplio (bacterias y hongos). La mayoría de los estudios previos sólo se han centrado en una de estas categorías taxonómicas, lo que hace que se pierda mucha información sobre las complejas relaciones sintróficas que pueden darse. La complejidad y el tamaño de la planta de compostaje estudiada es otro elemento de gran interés.

Palabras clave: composta, comunidades bacterianas y fúngicas, DGGE-PCR, estabilidad de la composta, PCR anidada.

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1 Introduction

In Mexico City, the quantity of waste generated by anthropogenic activities has been on the rise in recent years, in parallel with the growth of the population. A daily generation of 12204 t of municipal solid waste was registered in 2013 (Noyola et al., 2014), increasing to 12893 t in 2014 and 12816 t in 2015. According to the latest data from the Mexico City Solid Waste Inventory, approximately 12404 t of municipal solid waste (MSW) were generated daily in Mexico City in 2022, equating to an estimated generation of 1.07 kg d⁻¹ inhabitant⁻¹ (SEDEMA, 2022). Approximately 48% of this MSW is derived from households, 30% from services and businesses, 10% from markets, 5% from the Mexico City wholesale central market (Central de Abasto), 4% from various sources, and 3% is of controlled origin, including prisons, government offices, and medical units. The Ministry of Environment and Natural Resources (SEMARNAT, 2021) has reported that 38% of MSW is comprised of organic solid waste (OSW), which is classified into two major categories: pruning and gardening waste and food remains. The total quantity of organic solid waste generated per day is approximately 4676.3 t, with approximately 1374 t (over 30% of the MSW generated in Mexico City) received daily at the Bordo Poniente Composting Plant (BPCP, West Rim Composting Plant). The BPCP began its operation in 2012; the surface area of the facility is 370,000 m², with an installed capacity of 876000 t year-1, and a compost production of 89717 t year-1. Several works have been previously published regarding utilization of this Municipal Solid Organic Waste (MSOW) plant (Castilla-Hernández et al., 2016; Estrada-Martínez et al., 2021; Gan et al., 2013; Jimenez-Rodriguez et al., 2020). The facility comprises approximately 1000 piles, each measuring 200 m in length and 3 m in height. In consideration of the capacity of BPCP, it is the largest compost plant in Mexico and one of the largest in Latin America. In 2021, this composting plant received 449876 t of waste, which was transformed into 89702 t of compost. The quantity of organic solid waste (OSW) received daily (1374 t) for transformation into compost, along with the operational capacity of the plant (89 t d⁻¹) (SEDEMA, 2022), has led to the investigation of technologies that facilitate more efficient composting processes in recent years. In 2013, a waste separation program was established in Mexico City, where waste is collected from the source of generation (Programa General de Desarrollo Del Distrito Federal 2013-2018, 2013). Since a large portion of municipal solid waste (MSW) is generated in households, a segmented collection route was implemented where OSW is collected exclusively from households in Mexico City three days a week. After collection, the waste is weighed and transported directly to the BPCP. At the plant, the OSW is

mixed with pruning and tree trimming waste from green areas. The composting process is carried out in open-air piles, which allows for the process to be aerobic (although BPCP is located at 2,250 msnm, with partial pressure of O2 24% lower than at sea level, physiological processes are unaffected). The plant staff monitor the temperature to ensure the process reaches the thermophilic phase (60-70 °C). They also maintain the moisture of the piles by recirculating the leachate from the piles that have been processing for longer. The piles are periodically turned to prevent ignition zones and maintain homogeneity. Once the process is complete, the compost is screened to remove any remaining inorganic materials such as glass or plastic. After screening, the compost is placed in windrows for maturation.

Composting is defined as a biological process of aerobic degradation of organic matter through the succession of microbial communities of fungi and bacteria that have specific functions, resulting in the mineralization of easily degraded organic matter and the formation of a biologically stable end product (Noor et al., 2024; Xi et al., 2015; Yamamoto & Nakai, 2019). It is of great importance to understand the microorganisms present during the composting process. Nevertheless, technical challenges exist in conducting complex analyses based on direct isolation of microorganisms due to the potential for significant bias when selecting the culture media for isolation (Chen et al., 2023). Consequently, molecular tools and methods are employed to conduct microbial ecology studies in complex ecosystems or processes such as composting. Some of these tools include polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE), collectively known as PCR-DGGE. This is a molecular method for rapidly detecting changes in the microbial community or performing a comparative analysis of environmental samples, thereby providing information on the distribution and composition of the microbial species present (Tu Anh et al., 2018). Despite the fact that this technique has now been virtually displaced due to the cheapening of metagenomics studies by next generation sequencing (García-Uitz et al., 2024; Loreto-Muñoz et al., 2024), the DGGE technique remains in use for this purpose. Aydin et al. (Aydin et al., 2015) employed this technique to identify alterations in the microbial community present in compost bioreactors following the addition of varying antibiotic concentrations. In a study conducted by Xi et al. (Xi et al., 2015), the polymerase chain reactiondenaturing gradient gel electrophoresis (PCR-DGGE) method was employed to assess the influence of multistate inoculation on the composition and dynamics of the fungal and bacterial communities in a municipal solid waste composting process. Tu Anh et al. (Tu Anh et al., 2018) conducted a study to analyze and compare

Table 1. Identification codes and meteorological conditions during the sampling in BPCP in Mexico City.

Year	Month	Sample code	Season	Climate season	Precipitation* (mm*m ⁻²)	Mean Temperature (°C)	Maximum Temperature (°C)	Minimum Temperature (°C)
	March	MAR13	Spring	dry	0.0	14.7	24.4	5.0
2012	May	MAY13	Spring	rainy	1.3	18.4	27.5	9.2
2013	September	SEP13	Autumn	rainy	4.6	17.2	22.4	12.0
	October	OCT13	Autumn	rainy	1.4	16.5	23.5	9.4
	February	FEB14	Winter	dry	1.8	14.1	24.7	3.4
	March	MAR14	Spring	dry	0.8	16.0	25.3	6.8
	May	MAY14	Spring	rainy	2.1	17.2	25.2	9.1
2014	July	JUL14	Summer	rainy	3.6	17.1	24.0	10.3
	September	SEP14	Autumn	rainy	2.8	17.3	24.0	10.7
	October	OCT14	Autumn	rainy	4.9	15.5	22.4	8.5
	November	NOV14	Autumn	dry	0.1	14.2	22.6	5.9
	February	FEB15	Winter	dry	0.1	12.8	22.2	3.5
2015	March	MAR15	Spring	dry	3.1	15.0	22.8	7.2
2015	May	MAY15	Spring	rainy	4.4	17.8	25.5	10.0
	September	SEP15	Autumn	rainy	2.0	17.2	23.7	10.8

^{*} Average rainfall per month

the effectiveness of different sets of primers for the identification of fungi present in compost samples. Their findings indicated that, on certain occasions, DNA extractions may contain PCR inhibitors. Pandey et al. (Pandey et al., 2024) conducted a study in which they analyzed the evolution microbial communities and microbial shifts under anaerobic processes using DGGE. They applied this technique to analyze the dominant bacterial community in mesophilic and thermophilic anaerobic digestions of dairy manure in different temperatures (28-52 °C), examining the variable region V3 of the 16S rRNA gene of the present bacteria. The results showed that the bacterial community was affected by temperature and anaerobic incubation time, with significant changes in the microbial community.

To reduce the time required for composting processes, it is necessary to explore ways to modify the bioprocessing conditions to promote the specific activity of certain microorganisms. To achieve this, it is essential to have a comprehensive understanding of both the composting process conditions and the microorganisms involved. For this reason, the objective was to conduct a physicochemical and respirometric analysis of BPCP compost during the first three years of operation of the plant, period during both the dry and rainy seasons in Mexico City. The analysis was conducted to determine the stability of the compost and variation in the bacterial and fungal communities present, which was analyzed using the PCR-DGGE technique through the sequence of the predominant operational taxonomic units (OTUs).

2 Materials and methods

2.1 Sampling

A series of composting sampling procedures were conducted at the Bordo Poniente Composting Plant (BPCP) in Mexico City (19.461604451263426, -99.01750305249477), between 2013 and 2015. These procedures were conducted over several months during both the dry and rainy seasons, as detailed in Table 1. The sampling was conducted in accordance with the procedures outlined in the Test Methods for the Examination of Composting and Compost (TMECC), Method 02.01.(Thompson *et al.*, 2001) All samples were obtained from the mature compost storage site at the BPCP and were maintained at a refrigerated temperature until analysis.

2.2 Analytical techniques

2.2.1 Moisture determination

The moisture content was determined in triplicate by gravimetry using the standard method TMECC 03.09-A (Thompson *et al.*, 2001), as outlined in the Thermobalance OHAUS automatic moisture analyzer.

2.2.2 pH determination

The pH was determined using a calibrated potentiometer (Conductronic, model pH 120). In a beaker, 10 g of compost were weighed, and deionized water was added at a 1:5 ratio in accordance with the methodology outlined in section 04.11 of the TMECC

(Thompson *et al.*, 2001). This standard establishes the requirements for compost production from the organic fraction of urban, agricultural, livestock, and forestry solid waste and the quality specifications for compost produced and distributed in Mexico City (SEDEMA, 2011).

2.2.3 Carbon/Nitrogen (C/N) ratio determination

The C/N ratio was determined using a CHN elemental analyzer (Model 2400, Series II, Perkin Elmer). Between 2 and 3 mg of dried and sieved (mesh No. 100) compost samples were weighed in a tin capsule for analysis, in accordance with the manufacturer's instructions (Perkin Elmer Company, 2005). All samples were kept desiccated, and the analyses were performed in triplicate.

2.2.4 Soluble reducing sugar determination

In an Erlenmeyer flask, 5 g of the sample was weighed, and 20 mL of distilled water was added. The flasks were agitated on an orbital shaker at $21\,^{\circ}\text{C}$ for 10 min at 300 rpm. The samples were then centrifuged for three min at $16000 \times g$. One mL of the supernatant was then extracted and analyzed for soluble reducing sugars (RS) using the DNS method (Miller *et al.*, 1960). A volume of 1.5 mL of the DNS reagent was added to tubes containing 1 mL of the extract. The tubes were then placed in a boiling water bath for 10 min to develop color. Subsequently, the tubes were immersed in a cold-water bath, and the absorbance was read at 630 nm using a microplate reader (Elx808, BioTek).

2.2.5 Respirometric analysis

The compost samples were analyzed by respirometry in accordance with the procedure described by Martínez-Valdez *et al.* (2015) to quantify the production of CO₂ using a submerged system of packed-bed column bioreactors. The glass columns were filled with 30 g of compost, and the pH and humidity were not adjusted. During the analysis, a constant volumetric rate of 0.5 vkgm was maintained, and the analysis was performed over 48 h at 30 °C.

2.3 Molecular analysis techniques

The following techniques were used to perform PCR-DGGE analysis:

2.3.1 DNA extraction

DNA was extracted from biomass pellets (with a weight between 0.8 and 1.0 g) using a commercially available kit (PowerSoil® DNA Isolation Kit, MoBio Laboratories, Inc.). The integrity of the extracted DNA was evaluated through electrophoresis on a 1% agarose gel, which was run at 90 V for 40 min in 1X TAE buffer.

The concentration and purity of the obtained DNA were determined spectrophotometrically (NanoDrop 2000, Thermo Scientific). Additionally, the A_{260}/A_{280} ratio was determined to assess its suitability for amplification reactions.

2.3.2 Nested PCR

Two distinct regions (for bacteria and fungi) were subjected to phylogenetic analysis. The 16S rDNA gene was amplified for the bacteria, followed by nested PCR of the V6-V8 region of the same gene. For fungi, the genomic region encoding the ribosomal RNA precursor, including the internal transcribed spacers ITS1 and ITS2, was initially amplified. Subsequently, the internal transcribed spacer 1 (ITS1) of this region was amplified by nested polymerase chain reaction (PCR).

To identify bacteria, 16S rDNA amplification was conducted in an endpoint thermocycler (Multigene, Labnet International, Inc.). The primers employed for the amplification process were 8F (5' AGA GTT TGA TCC TGG CTC AG 3') and 1492R (5' TAC GGT TAC CTT GTT ACG ACT T 3') (Forney et al., 2004). The reaction mixture included 1.5 mM MgCl₂, 200 μM of each dNTP (Promega Corporation USA), 10 pM of each primer, 1.5 U of GoTag® Flexi DNA Polymerase (Promega Corporation USA) and 10-20 ng μ L⁻¹ of DNA template in 1X buffer. The amplification conditions were as follows: the initial denaturation phase was conducted for two min at 95 °C, followed by 25 cycles. Each cycle consisted of 30 s at 95 °C, 30 s at 50 °C for annealing, and one min at 72 °C for extension. A final extension step of five min at 72 °C was performed. The amplicons were subsequently subjected to analysis by 1% agarose gel electrophoresis (90 V, 45 min) stained with Texas Red® (Thermo Fisher Scientific). Following electrophoresis, the resulting bands were visualized under UV light using a transilluminator (Gel Logic 2200 Pro, Carestream Health, Inc.). Subsequent to the amplification of the 16S rDNA gene, nested PCR was performed to amplify the V6-V8 region with the previous product serving as the DNA template. The primers employed in this study were 1070F (5' ATG GCT GTC GTC AGC T 3') and 1392R+GC (5' GCC GCC CGC CCC GCG CCC GGC GCC GCC GCC CCC C ACG GGC GGR GRG CT 3') (Burr et al., 2006) The amplification conditions followed those previously established for the amplification of the 16S rDNA gene, with the exception of the annealing temperature, which was elevated to 55 °C. As previously stated, the visualization of the amplicons was conducted. The purified products were obtained using a commercial kit in accordance with the protocol described by the supplier (Wizard® SV Gel and PCR Clean-Up System, Promega Corporation). Subsequently, the concentration of the products was determined through spectrophotometric analysis.

In order to identify the fungi present, a preliminary amplification reaction was conducted using the primers ITS1 (5' TCC GTA GGT GAA CCT GCG G 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') (White et al., 1990). The reaction mixture consisted of 1X PCR Super Master Mix (Biotools, B & M Labs, S.A.), 10 pM of each primer and 1-20 ng of template DNA. The amplification conditions were standardized as follows: an initial denaturation for 3 min at 95 °C. was followed by 30 cycles, each consisting of 30 s at 95 °C denaturation, 30 s at 60 °C annealing, 30 s at 72 °C extension, and a final extension for 2 min at 72 °C. The second amplification reaction was conducted using the previous products as template DNA with the primers ITS2 (5' GCT GCG TTC TTC ATC GAT GC 3') and ITS5+GC (5' CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAA GTA AAA GTC GTA ACA AGG 3'). The reaction mixture and amplification conditions were identical, with the exception of the annealing temperature, which was elevated to 65 °C.

2.3.3 DGGE analysis

The amplicon profiles were analyzed using a vertical electrophoresis chamber (DCode Universal Mutation Detection System, Bio-Rad Laboratories) on 7% acrylamide:bisacrylamide (37.5:1) with a parallel denaturing gradient, ranging from 30-65% for bacterial analysis and 25-55% for fungal analysis. The electrophoresis was conducted for 19 h at 60 °C and 80 V in 0.5X buffer (Neilson et al., 2013). Each gel lane was loaded with 100 ng of purified amplicons from nested-PCRs. Following electrophoresis, the acrylamide gels were silver stained using the technique described by Sanguinetti et al. (1994). The obtained gels were digitized using a photo documenter (MiniLumi, DNR Bio-Imaging Systems) and the band analysis was conducted using GelQuant software (DNR Bio-Imaging Systems). Subsequently, selected bands were excised and reamplified using the aforementioned primers, namely 1070F and 1392R for bacteria and ITS2 and ITS5 for fungi, respectively, in the absence of the -GC clamp. The amplification conditions were as previously described. The purified products were then subjected to further analysis using the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation). After that, the samples were quantified and prepared for subsequent sequencing.

2.3.4 Sequencing and bioinformatic analysis

The purified samples were subsequently sent to Macrogen, Inc. (Seoul, Korea) for sequencing. Sequence analysis was conducted after the sequences were edited using the bioinformatics programs Clustal X V. 2.1 (http://www.clustal.org/clustal2/) and BioEdit V. 7.2 (https://thalljiscience.

github.io/). The sequences were compared with the GenBank 16S rDNA and ITS1 gene databases using BLASTn, with uncultured/environmental sample sequences being excluded.

The nucleotide sequences included in this work were deposited under Bioproject PRJNA1047156 (accession numbers: KIDT00000000 for 16S rRNA and KIDU00000000 for ITS) as a Targeted Locus Study project in DDBJ/ENA/GenBank.

3 Results and discussion

3.1 Climatological conditions during compost sampling

The identification codes of the 15 samples and meteorological conditions during the sampling in BPCP in Mexico City are presented in Table 1. Four samples were collected in 2013, seven in 2014, and four in 2015. In terms of temporal distribution, samples were obtained from the four seasons of the year, with the highest frequency observed in summer and autumn. The average, maximum, and minimum temperatures, as well as the evaporation rate, were obtained from the database of the National Meteorological System of Mexico (SMN). The data were specifically extracted from the meteorological station of Nabor Carrillo Lake (Code C15383), situated in the Municipality of Texcoco de Mora (State of Mexico), approximately 5 km from the BPCP. The months with the highest rainfall were September 2013, October 2014, and May 2015, with 4.6, 4.9, and 4.4 mm m⁻², respectively. The minimum temperature for the months of sampling was maintained at intervals between 3.4 and 12 °C, the maximum temperature was between 22.2 °C (February 2015) and 27.5 °C (May 2013), and the average temperature recorded by the meteorological station was between 12.8 and 18.4 °C. It is noteworthy that in Mexico City, the period of lower rainfall occurs from December to April. During this period, precipitation levels decline, resulting in drier conditions (Table 1). In 2014, the BPCP implemented a modernization redesign in the composting process and a change in the location of the finished product for stabilization. Moreover, the final compost was placed in an open environment, which favored humidification due to rainfall during the rainy season and dehydration during the hot months. This resulted in differences in the final characteristics of the product. During the process of modifying the BPCP, a dome was constructed to maintain the maturity of the compost throughout the year.

3.2 Physicochemical and respirometric analysis of compost

The moisture content (in percent) of all compost samples ranged from 10.3 % \pm 0.2 to 46.5 % \pm 0.7, with notable fluctuations observed across different months of the year. The samples from March, May, and June of the three years (except for May 2014) exhibited lower moisture contents, while all the samples from September exhibited the highest moisture content. This high moisture content corresponded to the rainy season in Mexico City, as indicated in Table 1. According to the Mexican standard (NADF-020-AMBT-2011) (SEDEMA, 2011), the moisture content of the compost must be between 25 and 45 % by weight. Based on this parameter, it can be concluded that all the samples analyzed correspond to stable and good-quality compost. The concentration of soluble reducing sugars (RS) in the samples showed significant variations, regardless of the sampling month. The samples from March exhibited the highest concentration of reducing sugars, followed by those from May, which corresponded to the spring season. During this period, many of the organic residues found in the BPCP originated from fruits with high sugar contents.

Conversely, studies evaluate compost quality by determining maturity and stability, yet fail to clearly define the parameters to be used to determine these conditions (Azim *et al.*, 2017; Olfa, 2013). Azim *et al.* (Azim *et al.*, 2017) proposed a division into three categories, depending on the process stage. The initial category encompasses the proposed starting parameters, including the C/N ratio, moisture content, porosity, and particle size. The second category encompasses parameters that are monitored during the composting process, including temperature, oxygen consumption

and CO_2 production, enzyme activities, and organic carbon. The third category assesses the quality of the final product by parameters such as the C/N ratio, humic substances, electrical conductivity (CEC), and salinity.

The effects of various factors, including pH, moisture content (%), the C/N ratio, reducing sugars, and CO₂ production rate, on the quality of the BPCP compost were analyzed (Table 2). The pH values of all samples were found to be between 8 and 9, indicating that all samples were obtained from composting piles that had reached an advanced stage of curing or stabilization (Barrena et al., 2009; Kumar Singh et al., 2009; Rastogi et al., 2020). The C/N ratio of each sample was less than 12, except for that of compost SEP14, which had a value of 13.6 ± 0.5 . These results indicated that the compost in the sampled piles had already reached a stable stage, which corroborated with the findings of other authors (Guo et al., 2012; Xiao et al., 2009) and with the Mexican standard NADF-020-AMBT-2011 (SEDEMA, 2011).

Regarding the maximum CO_2 production rate (Table 2), all samples exhibited values within the range of 0.01 ± 0.001 and 3.48 ± 0.32 mg CO_2 g⁻¹ IDM h⁻¹. The moisture content (%), pH, C/N ratio and CO_2 production rate of all the samples studied were within the limits for stable compost according to the local and international standards of the NADF-020-AMBT-2011 (SEDEMA, 2011) and US Composting Council (US Composting Council, 2023). Furthermore, no significant differences were found between the BPCP compost samples. The results presented indicate that the technical modifications implemented in the BPCP during 2014 were effective in maintaining the quality of the compost obtained.

Table 2. Physicochemical analysis and maximum CO₂ production rate of compost samples from BPCP in Mexico City.

Year	Sample code	Moisture (%)	pН	C/N ratio	Reducing sugars (mg g ⁻¹ IDM)	Maximum CO ₂ production rate (mg CO ₂ g ⁻¹ IDM h ⁻¹)
2013	May-13 Sep-13 Oct-13	12.6 ± 0.3 38.9 ± 1.1 36.0 ± 1.9	8.2 ± 0.1 8.9 ± 0.0 8.9 ± 0.1	9.5 ± 0.4 11.7 ± 0.5 8.5 ± 0.2	4.0 ± 0.2 1.0 ± 0.2 0.6 ± 0.1	0.03 ± 0.01 2.92 ± 0.03 0.09 ± 0.01
2014	Feb-14 Mar-14 May-14 Jul-14 Sep-14 Oct-14 Nov-14	18.5 ± 1.0 11.2 ± 0.3 30.5 ± 0.6 10.5 ± 0.3 46.5 ± 0.7 16.3 ± 0.3 18.3 ± 0.3	8.7 ± 0.2 8.4 ± 0.2 8.8 ± 0.2 8.9 ± 0.1 8.4 ± 0.0 8.5 ± 0.1	9.8 ± 0.4 9.7 ± 0.3 8.1 ± 0.1 8.9 ± 0.1 13.6 ± 0.5 9.4 ± 0.9 9.2 ± 0.2	0.6 ± 0.1 6.0 ± 0.1 4.0 ± 0.3 0.4 ± 0.0 3.1 ± 0.1 1.9 ± 0.1 2.2 ± 0.2	0.51 ± 0.06 0.05 ± 0.01 0.02 ± 0.01 0.02 ± 0.01 3.48 ± 0.32 0.01 ± 0.01 1.20 ± 0.02
2015	Feb-15 Mar-15 May-15 SEP15	15.0 ± 0.3 12.0 ± 0.4 11.3 ± 0.6 28.6 ± 1.0	8.7 ± 0.0 8.7 ± 0.2 8.9 ± 0.0 8.9 ± 0.1	9.1 ± 0.2 9.3 ± 0.2 10.4 ± 0.4 9.0 ± 0.2	1.0 ± 0.1 5.9 ± 0.6 3.5 ± 0.4 0.6 ± 0.1	0.04 ± 0.01 0.01 ± 0.01 0.03 ± 0.01 1.23 ± 0.02

Table 3. Bacteria identified from BPCP in Mexico City. The asterisk indicates the samples in which each of the bacteria was detected. The percentage of identity is expressed in comparison to the NCBI database.

Microorganism	Identity		Sample code													
	(%)		2013				2014						2015			
		MAR	MAY	SEP	OCT	FEB	MAR	MAY	JUL	SEP	OCT	NOV	FEB	MAR	MAY	SEP
Acinetobacter albensis (2)	100	*	*		*	*	*					*	*	*	*	
Acinetobacter equi (2)	> 98.7	*	*	*	*	*	*									*
Acinetobacter johnsonii (12)	> 99.4	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Acinetobacter sp. (12)	> 96.6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Bacillus benzevorans	99	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Bacillus sp.	95.7	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cutibacterium sp.	97.7	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Porticoccus sp.	96	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pseudomonas sp.	98.2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Saccharopolyspora sp.	97.1	*	*					*	*							
Variovorax sp.	95.1			*	*	*	*					*	*	*	*	*
Xanthomonas sp.	96	*														

3.3 Analysis of bacterial communities by PCR DGGE

High-molecular-weight DNA was successfully extracted from all samples, with an absorbance ratio (A_{260}/A_{280}) close to 1.8, indicating that the DNA was relatively pure (Wilfinger et al., 1997). According to the primers used, the fragments obtained via nested PCR had an approximate length of 300 bp (Piterina & Pembroke, 2013). The bacterial profiles of the different compost samples obtained during the three years were studied. Image analysis (Figure 1S, supplementary material) yielded 233 total bands distributed among the 15 compost samples. A total of 59 bands were selected and cut based on their ease of cutting, representativeness, and intensity as observed during the development of the gel (Figure 1S, supplementary material). Following the bioinformatic analysis, data was obtained from 36 operational taxonomic units (OTUs), corresponding to 61% of the selected bands.

One of the most crucial aspects of studying composting from a microbial perspective is the diversity of fungi and bacteria present in piles during the composting process. This diversity is crucial for the degradation of organic matter. Consequently, considerable effort has been invested in recent years to study these microorganisms with the objective of enhancing their degradation efficiency (Maeda *et al.*, 2010; Rastogi *et al.*, 2020).

Studies of microbial diversity constitute a sound experimental strategy. Nevertheless, diversity is frequently constrained by the quantity and quality of organic matter available for compost production. This in turn is influenced by the physicochemical characteristics of the compost at the end of the composting process (Francou *et al.*, 2007; Rastogi *et al.*, 2020). Table 3 presents the bacteria identified in this study. Some of the sequences analyzed exhibited a percentage of identity above 98.5 %, indicating that they could be assigned to the species level. In contrast, the remaining sequences exhibited a percentage of

identity below the species level threshold, and thus could only be assigned to the genus level. The compost samples exhibited a similar diversity, except for those obtained in 2014, which exhibited a notable decline in the number of bacteria present. This decline can be attributed to the disturbances that occurred during the redesign of the BPCP, which affected the microbial diversity but not the final stability and maturity characteristics of the compost.

Some identified bacteria were intermittently present, including Saccharopolyspora sp. (previously known as Streptomyces sp.), which has an industrial application in the production of erythromycin and has frequently been found in soil (Saygin et al., 2021), and Variovorax sp., which produces octopin, a chemical compound related to the formation of tumors in the roots of some plants (Han et al., 2016) and to the degradation of phthalates, which are compounds added to plastics to increase their flexibility (Prasad & Suresh, 2012). This issue may be related to the presence of plastics of different types mixed with the organic matter that is destined for composting. During the separation of the municipal solid waste, the plastic materials are not completely removed from the organic fraction of the municipal solid waste. Seven of the 12 identified bacteria (Acinetobacter johnsonii, Bacillus benzoevorans, Acinetobacter sp., Bacillus sp., Cutibacterium sp., Porticoccus sp. and Pseudomonas sp.) were found in all the compost samples, which may suggest that these bacteria are very well adapted and established in the BPCP ecosystem and play an essential role in the degradation of the organic matter. The presence of these genera has also been reported in compost derived from a variety of materials (Aydin et al., 2015; Bouhia et al., 2022; Horisawa et al., 2008; Sundberg et al., 2011; Zhang et al., 2014). This is likely due to their ability to produce hydrolytic enzymes, including hemicellulases, cellulases, proteases, and lipases (Table 4), which are closely related to organic matter degradation.

Table 4. Major characteristics of the bacteria identified in the BPCP in Mexico City.

Microorganism	Major characteristics of the bacteria identified Origin and enzyme production	References
Acinetobacter albensis	Isolated from bodies of water, surfaces of stored fish, and commonly found in soil. Produce proteases.	Al Atrouni <i>et al.</i> , 2016; Kaszab <i>et al.</i> , 2021; Krizova <i>et al.</i> , 2015
Acinetobacter equi	Horse faeces and soil. Bacteria capable of reducing nitrates, utilizing L-arabinose, caproate, malate, citrate and producing organic acids from D-glucose.	Al Atrouni et al., 2016; Poppel et al., 2016
Acinetobacter johnsonii	Isolated from activated sludge, bodies of water, . stored fish, wastewater, soils contaminated with pesticides, food and compost	Al Atrouni et al., 2016; Kaszab et al., 2021
Acinetobacer spp.	Water, soil, meat, fish, shrimp, sediments, plant nectar, animals, farms, biogas plants, aerobic digesters and contaminated soils.	Al Atrouni et al., 2016; Pulami et al., 2023
Bacillus benzoevorans	Isolated from soils, landfills. Degradation of phenolic compounds, hemicellulases, laccases, ammonium, and sulfate-reducing enzymes.	Madani <i>et al.</i> , 2021
<i>Bacillus</i> sp.	Isolated from soil, water, decomposing plant material, industrial and extreme environments. Used in inoculum for the degradation of plant organic matter. Herbicide degrader produces α -amylases, alkaline proteases, lipases, cellulases.	Qian et al., 2023; G. Zhao et al., 2023
Cutibacterium sp.	Present in the rhizosphere of healthy soils. The presence of phytopathogenic fungi stimulates its presence in diseased soils.	T. Lin et al., 2023
Porticoccus sp.	Found in mangrove sediments, a phenanthrene and hydrocarbon-degrading microorganism.	Gao et al., 2022; Tiralerdpanich et al., 2021
Pseudomonas sp.	Degradation of aromatic hydrocarbons, phenols, plant growth-promoting rhizobacteria (PGPR), biocontrol, plant and animal pathogen, isolated from soil and compost. Cellulases and lipases.	Azhdarpoor et al., 2014
Saccharopolyspora sp.	Isolated from soil. Some species can degrade complex polymers such as starch and xylan. Antibiotic production.	Oliynyk et al., 2007; Saygin et al., 2021
Variovorax sp.	Isolated from soil. Capable of degrading phthalates, polylactic acid, polyhydroxyalkanoates. Produces cellulases, proteases.	Han et al., 2016; Prasad & Suresh, 2012
Xanthomonas sp.	Plant pathogen, soil isolates, insect gut isolates. Degradation and bioconversion of polyurethane (polyacrylic, polyester and polyether).	Kim <i>et al.</i> , 2022; Rahman <i>et al.</i> , 2014

Acinetobacter equi was present in all the samples collected between March 2013 and March 2014. It was subsequently identified in a sample collected in September. Acinetobacter spp. is worthy of particular attention, given that they have been isolated from a variety of compost samples (Sundberg et al., 2011). They have been reported to be a bacterium capable of degrading oils and acting as an adjuvant in the bioremediation of soils (C. Lin et al., 2022; Van Gestel et al., 2003). Additionally, they can partially degrade low-molecular-weight polyethylene oligomers, a precursor material for plastics. This genus has been identified in a considerable number of the different OTUs selected (27 in total), potentially due to the

variable number of copies of the ribosomal operon per bacterial genome, which can range from one to 15 copies, with seven copies observed in the case of *Acinetobacter* sp. (del Rosario Rodicio & del Carmen Mendoza, 2004) as the copies differ in sequence, they can be detected as different OTUs.

Given the paucity of studies in this area, a comparison with the only available work in which the microbial diversity of the BPCP was analyzed is essential to contextualize our findings, despite the evident differences in terms of temporality and sample type (mature compost vs. leachates) and in the microbial groups analyzed (bacteria and fungi vs. bacteria and archaea). The finding that, in both cases,

the presence of bacteria of the genus *Acinetobacter* was found in higher proportion in all samples analyzed (Gállego Bravo *et al.*, 2019) is of particular interest.

3.4 Analysis of fungal communities by PCR DGGE

Figure 2S (supplementary material) presents the DGGE profiles for fungi obtained for each of the compost samples under study. A total of 52 OTUs were selected from 250 bands identified by the GelQuant program. A total of 24 OTUs were identified, although in some instances, the same information was provided by different OTUs due to the DGGE technique detecting mutations that may result in changes to the position of the bands detected in comparison to wild types (Strathdee & Free, 2013). Table 5 presents the identified fungi in each compost sample, including the identity (%) for each of the sequences analyzed. Similarly, as in the analysis of bacteria, fungi could be assigned at the species level following the attainment of a percentage of identity exceeding 98.5 %. Conversely, those samples exhibiting lower values below the genus level were assigned. Three fungal species were consistently detected in all samples, namely Botryotrichum sp., Metarhizium anisopliae and Peniophora sp. In contrast, the remaining fungal species exhibited intermittent detection. Some fungi were detected at three consecutive sampling times, while others were not. This was the case for Aspergillus niger and Thermomyces dupontii, which appeared in the same samples. Preussia sp. was not detected in the initial samples. It was first observed in the NOV14 sample, after which it was consistently present in all subsequent samples. Its appearance may be associated with the modernization and standardization of the composting process that occurred in 2014, as previously mentioned. In the three samples collected in

March, the following fungi were detected: Aspergillus flavus, Botryotrichum sp., Metharhizium anisopliae, Peniophora sp., and Scopulariopsis sp. This indicates that some of the previously identified fungi persisted. Table 6 provides a detailed description of the major characteristics of the identified fungi. Regarding their provenance, numerous reports have indicated that a significant proportion of these fungi have been identified in soil, as part of the wider ecosystem, or as plant endophytes. Nevertheless, research has indicated that these fungi can cause disease in plant species, including Aspergillus flavus, Exophiala sp., Fusarium sp. and Scopulariopsis sp. All the fungi identified in this study have been extensively studied as producers of extracellular hydrolytic enzymes, which are related to organic matter degradation. This is because they are found in sites with abundant organic matter that can be used as a carbon and nitrogen source. Some fungal genera warrant particular attention, including Aspergillus niger, Diutina rugosa and Thermomyces dupontii. These have been extensively studied and present biotechnological potential for use in several industrial sectors (Christopher et al., 2005; C. Lin et al., 2022; Sonia et al., 2005).

Conclusions

Our results contribute to understand the dynamics of microbial communities in complex systems such as composting. It is important to note that the stability of microbial communities in plants or sites operated for long periods is a phenomenon that is influenced by different environmental factors and operational variables such as turning, aeration, humidity and temperature control, among other factors. This makes some of these sites subject to constant disturbances.

Table 5. Identified fungi in each of the samples obtained from the BPCP in Mexico City. An asterisk indicates the samples in which each of the fungi was detected. The percentage of identity is expressed in comparison to the NCBI database.

Microorganism	Identity							9	Sample							
	(%)		201	13					2014					20	15	
		MAR	MAY	SEP	OCT	FEB	MAR	MAY	JUL	SEP	OCT	NOV	FEB	MAR	MAY	SEP
Aspergillus flavus (3)	> 98.8	*		*	*	*	*	*	*	*		*	*	*	*	*
Aspergillus niger (1)	100	*	*	*		*	*	*		*	*	*	*			*
Botryotrichum sp. (3)	> 97.6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Diutina rugosa (3)	> 98.5	*		*						*	*		*	*	*	
Exophiala sp.	95.2			*			*			*			*	*		*
Exserohilum rostratum	98.4			*								*	*		*	
Fusarium sp.	100	*			*	*				*						
Gymnascella aurantiaca	97.8		*				*	*	*						*	
Metarhizium anisopliae	100	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Peniophora sp. (3)	>98.4	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pichia kudriavzevii	99.4	*		*						*	*					*
Preussia sp.	95.2											*	*	*	*	*
Scopulariopsis sp.	97.9	*	*	*	*	*	*			*		*	*	*	*	*
Thermomyces dupontii (2)	> 98.8	*	*	*		*	*	*		*	*	*	*			*
Thermomyces sp.	98.2		*				*	*	*						*	

Table 6. Major characteristics of the fungi identified in the BPCP of Mexico City in this study.

Microorganism	Origin and enzyme production	References
Aspergillus flavus	Isolated from soil. Plant pathogen. Produces aflatoxins, polygalacturonases, laccases and xylanases.	(de Alencar Guimaraes et al., 2013)
Aspergillus niger	Soil fungus used in second-generation biofuel production. Hemicellulases, pectinases and xylanases.	(Gomes et al., 2011)
Botryotrichum sp.	Isolated from pig manure compost, heavy metal passivation produces cellulases. Antibiotic synthesis in compost.	(Wu et al., 2022; B. Zhao et al., 2023)
Diutina rugosa	Production and immobilization of lipases; isolated from soil and oral cavities.	(Barton, 2011)
Exophiala sp.	Aquatic environments, pathogen of aquatic plants and animals. Chitinases and keratinases.	(do Nascimento et al., 2016)
Exserohilum rostratum	Endophyte of orchids, causing mycosis in humans.	(Chua et al., 2022)
Fusarium sp.	Isolated from plants and soil, plant pathogens and endophytes. Cellulases and hemicellulases.	(Chua <i>et al.</i> , 2022; Kashiwa <i>et al.</i> , 2017)
Gymnascella aurantiaca Metarhizium anisopliae	Isolated from soil. Production of keratinases. Entomopathogen of plants, found in soil. Produces lipases, hemicellulases.	(Scott & Untereiner, 2004) (Aw & Hue, 2017)
Peniophora sp.	Isolated from wood, plant pathogen. Produces hemicellulases, cellulases.	(Lee et al., 2015)
Pichia kudriavzevii	Yeast isolated from fermented fruits. Used for second-generation bioethanol production.	(Hoppert <i>et al.</i> , 2022)
Preussia sp.	Present in soil, activator of nitrogen variation inside cow manure compost. Producer of phytohormones, phosphatases and glucosidases.	(Khan et al., 2016; Sun et al., 2022)
Scopulariopsis sp.	Soil, plant material and insects; wheat cultivation fields and soil, plant pathogen. Produces cellulases, keratinases and chitinases.	(Sharaf & Khalil, 2011; Skóra <i>et al.</i> , 2014)
Thermomyces dupontii	Soil, compost; plant pathogen. Produces chitinases, glucosidases and xylanases.	(Nisar <i>et al.</i> , 2022)
Thermomyces sp.	Soil; production of xylanase, hemicellulases, lipases enzymes.	(Sun et al., 2022)

Furthermore, the composition of municipal solid waste in large cities such as Mexico City and its Metropolitan Zone is characterized by significant heterogeneity, resulting from a combination of socioeconomic, demographic and seasonal factors. All the samples collected were found to meet the criteria for classification as stable compost according to Mexican and international standards. A total of 111 operational taxonomic units (OTUs) were identified in 15 compost samples. Eight bacterial genera and 15 fungal genera were observed, suggesting a greater fungal diversity than bacterial diversity. Nine microorganisms (Acinetobacter spp., Bacillus sp., Cutibacterium sp., Pseudomonas sp., Porticoccus sp., Botryotrichum sp., Metarhizium anisopliae and Peniophora sp.) were present in all the samples, indicating that these microorganisms are adapted and distributed in that ecosystem. All the identified microorganisms exhibited enzymatic activities related to organic matter degradation, with a clear preference for plant-derived substrates.

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Nomenclature

16S rDNA	Ribosomal 16S RNA gene
1X	Final stock concentration
A_{260}/A_{280}	Absorbance ratio
BLAST	Basic Local Alignment Search

Tool

BPCP	Bordo Poniente Composting
	Plant (West Rim Composting
	Plant)
C/N	Carbon/Nitrogen ratio
DGGE	Denaturing gradient gel
	electrophoresis
DNA	Deoxyribonucleic acid
DNS	Dinitro salicylic acid
DM	Dry matter
$\times g$	Gravity
IDM	Initial dry matter
ITS	Internal transcribed spacer
NCBI	National Center for
	Biotechnology Information
OTU	Operational taxonomic unit
OSW	Organic solid waste
RS	Reducing sugars
t	Metric ton (1000 kg)
TAE	Tris-acetate-EDTA
TMECC	Test methods for the examination
	of composting and compost

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