Vol. 24, No. 3(2025) Bio25551
Revista Mexicana de Ingeniería Química

Biodegradation potential of thermophilic cellulolytic bacteria isolated from urban organic waste composting

Potencial de biodegradación de bacterias celulolíticas termófilas aisladas del compostaje de residuos orgánicos urbanos

A. Avila-Andrade[1], R. Beristain-Cardoso[2], D. García-Mondragón[1], S. Alcaraz-Ibarra[1], J. F. Aguirre-Garrido[3], G. González-Blanco*

[1] Instituto Interamericano de Tecnología y Ciencias del Agua, Universidad Autónoma del Estado de México, Carretera Toluca-Atlacomulco km 14.5, Unidad San Cayetano, Toluca C.P. 50295, México.

[2] Department of Environmental Sciences, Metropolitan Autonomous University-Lerma. Av. de las Garzas #10, El Panteón, 52005 Lerma de Villada, State of Mexico, Mexico.

[3] Department of Earth Resources, Metropolitan Autonomous University-Lerma. Av. de las Garzas #10, El Panteón, 52005 Lerma de Villada, State of Mexico, Mexico.

Sent date: March 17, 2025; Accepted: July 9, 2025

Abstract

The composting process of urban organic waste presents a microbial diversity depending on the phases of the process, which makes it a source of isolation of thermostable and hydrolytic bacteria of biotechnological importance due to their ability to degrade complex compounds such as cellulose, hemicellulose, and lignin. The objective of this study was to isolate and characterize thermophilic cellulolytic bacteria, as well as to determine their hydrolytic and degradation potential. Microbial isolation was performed from compost samples in the thermophile phase (47°C ± 2.25). Identification was performed by biochemical tests and massive amplicon sequencing. Hydrolytic capacity was determined by hydrolysis halos and kinetic activity in 0.1% CMC medium at 50°C. *Bacillus sp*. and *Bacillus licheniformis* strains were isolated from a thermophilic-phase composting system. These strains exhibited specific growth rates of 0.1051 h^{-1} and 0.0794 h^{-1}, doubling times of 6.5 h and 8.8 h, and hydrolysis halo diameters of 1.08 cm and 0.8 cm, respectively. The identification of bacteria of the Bacillus genus from the composting process highlights its importance as a source of thermophilic bacteria with hydrolytic capacity for biotechnological applications.

Keywords: Isolation, composting, thermophilic, bacilli, cellulose, residue.

Resumen

El proceso de compostaje de residuos orgánicos urbanos presenta una diversidad microbiana en función de las fases del proceso, lo que lo convierte en una fuente de aislamiento de bacterias termoestables e hidrolíticas de importancia biotecnológica debido a su capacidad para degradar compuestos complejos como celulosa, hemicelulosa y lignina. El objetivo de este estudio fue aislar y caracterizar bacterias celulolíticas termófilas, así como determinar su potencial hidrolítico y de degradación. El aislamiento microbiano se realizó a partir de muestras de compost en fase termófila (47 °C ± 2.5). La identificación se realizó mediante pruebas bioquímicas y secuenciación masiva de amplicones. La capacidad hidrolítica se determinó mediante halos de hidrólisis y actividad cinética en medio CMC al 0.1 % a 50°C. Las cepas de *Bacillus sp*. y *Bacillus licheniformis* se aislaron de un sistema de compostaje en fase termófila. Estas cepas presentaron tasas de crecimiento específicas de 0.1051 h^{-1} y 0.0794 h^{-1}, tiempos de duplicación de 6.5 h y 8.8 h, y diámetros de halo de hidrólisis de 1.08 cm y 0.8 cm, respectivamente. La identificación de bacterias del género Bacillus en el proceso de compostaje resalta su importancia como fuente de bacterias termófilas con capacidad hidrolítica para aplicaciones biotecnológicas.

Palabras clave: Aislamiento, compostaje, termófilo, bacilos, celulosa, residuo.

* *Corresponding author. E-mail*: gehovan25@yahoo.com.mx;
https://doi.org/10.24275/rmiq/Bio25551
ISSN:1665-2738, *issn-e*: 2395-8472

1 Introduction

Composting is an aerobic and exothermic process where a complex microbial community carries out the biotransformation of organic matter (Moreno *et al*., 2021; Firone *et al*., 2023). Globally, 19% of urban solid waste was recycled or composted (Cao *et al*., 2023). Babu *et al*. (2021) indicated that composting is the most applied biotechnology, due to its ease of implementation and low cost. However, it has the major disadvantage of requiring long operating times (from 4 to 6 months, attributed to the presence of lignocellulosic material, pruning and gardening remains) to obtain mature compost (Waqas *et al*., 2023).

In addition, due to the large volume of waste, strategies to improve the process are being sought (de Mendonça *et al*., 2021). In this sense, techniques have been implemented to shorten composting times by improving control parameters, such as aeration, turning frequency (Getahun *et al*., 2012), optimization of the C:N ratio (Guo *et al*., 2012; Nguyen *et al*., 2020), and the type of bulking agent (carbon source). These improvements aim to produce compost suitable for soil enhancement or use as a biofertilizer (de Mendonça *et al*., 2021).

Currently, one approach consists of the bioaugmentation of microorganisms isolated from the same process with specific metabolic characteristics that can be used to accelerate the degradation process (Babu *et al*., 2021), as well as in other production processes (Zhu et al., 2020). The metabolic reactions of the communities present in the composting process generate energy and cause an increase in temperature in the system leading to four phases: mesophilic (20-40 °C), thermophilic (40-60 °C), cooling (40-20 °C), and maturation (room temperature) (Moreno *et al*., 2021). The increase in temperature during the thermophilic phase provides favorable conditions for the isolation of thermotolerant microorganisms (Ince *et al*., 2020), capable of growing at temperatures between 40 – 120 °C (thermophilic microorganisms), which produce thermostable enzymes, proteins that retain their structure and function at high temperature (Kanekar & Kanekar, 2022). The ability of thermostable enzymes to operate at high temperature is essential for the transformation of organic waste, a process of great relevance in areas such as waste management and biofuel production (Banerje *et al*., 2020; Hussian & Leong, 2023).

Thermophilic cellulolytic bacteria can be found in various environments that present extreme temperature conditions such as: dry tropical forests, deep-sea hydrothermal vents, geothermal areas, and in artificial systems such as waste treatment plants and compost piles (Kanekar & Kanekar, 2022; Firone *et al*., 2023). Authors such as López *et al*. (2021) stated that the microbiota present in composting process includes thermophilic microorganisms capable of degrading lignocellulose-rich residues. These microorganisms are of great biotechnological interest due to their resistance to chemical denaturation, their wide optimal pH range, and their broad substrate specificity (Hussian & Leong, 2023). Additionally, bacteria have shorter doubling times measured in days, compared to fungi, which may take weeks to double (Wang & Kuzyakov, 2024); these characteristics make bacteria valuable in industrial and biotechnological applications (Banerjee *et al*., 2020) such as bioremediation, biorefinery and bioplastic production (Li & Huo 2025).

Authors such as Siu-Rodas (2018) reported the isolation of three thermophilic strains (optimal growth at 60 ° C) capable of growing in acidic pH (4-5) with endocellulase and exocellulase activity and mentioned that these strains have a potential application in the extraction and clarification of juices as well as paper bleaching. Anguiano (2019) has isolated compost bacteria from organic waste in the thermophilic phase (e.g., *Bacillus pumilus, Stenotrophomonas* and *Bacillus subtilis*) which showed growth in a selective carboxymethylcellulose medium. While Finore *et al*. (2023) reported the isolation of lignocellulosic bacteria from sawdust and bovine manure compost, associated with the thermophilic phase of the process (55°C). Although the isolation of lignocellulosic bacteria is reported in the literature, few reports are available regarding their metabolisms, kinetics and doubling times with carboxymethylcellulose as the sole carbon source. Therefore, this study evaluated the isolation and phenotypic, genotypic, and metabolic characterization of cellulolytic thermophilic bacteria from composting.

2 Materials and method

2.1 *Obtaining composting samples*

The compost used in this study was produced from urban organic waste (a heterogeneous mixture of fruit peels, raw and cooked vegetables, green leaves, and food scraps) combined with black soil at a 2:1 ratio. The mixture was placed in a sealed container measuring 32 cm in width, 53 cm in length, and 26.5 cm in depth, equipped with a hermetic lid and a leachate collection system.

Composting was performed in duplicate with daily turning to ensure proper aeration. Each compost bin was loaded with 2.9 kg of black soil as a bulking agent (initial moisture content: 30%) and 5.8 kg of urban organic waste (initial moisture content: 85%). The temperature and pH were monitored with an electronic soil meter (4 in 1 Soil Survey Instrument) and the moisture with a Tempo Disc ™ Bluetooth Sensor for temperature. Chemical oxygen demand (COD)

was measured using the closed reflux method with HACH® COD reagent vials (Method 8000), following the procedure outlined in the Mexican standard NMX-AA-52-1985 (SEMARNAT, 1985). Inorganic nitrogen species were measured using the HACH Kit 8039 for nitrate, the HACH Kit 8507 for nitrite, and an ammonium-selective electrode (Phoenix Electrode Company, USA) for NH_4^+ quantification. The samples for isolation were taken from the thermophilic phase of the composting system on day 80, when it presented an average temperature of 47°C ± 2, moisture of 67.8 ± 0.51%, and pH of 8.32 ± 0.13.

2.2 *Microbial isolation*

During the thermophilic phase, 5% of the compost was sampled. From this portion, a 5 g sample was taken to activate the microorganisms in 45 ml of sterile nutrient broth (DB BIOXON®). The sample was then incubated at 50°C for 24 hours. Subsequently, 1 ml of activated culture was inoculated into nutrient agar plates using the pour-plate technique and incubated for 48 hours at 50°C. Colonies with distinct morphologies were then re-streaked into nutrient agar plates using the cross-streak method to characterize their morphology. Additionally, the culture was plated on carboxymethylcellulose (CMC) agar to isolate bacteria with cellulolytic activity. These plates were incubated at 50°C for 48 hours (Lynd *et al*., 2022).

2.3 *Macroscopic and microscopic characterization*

The macroscopic morphological characterization of the strains was carried out following the guidelines provided in Bergey's Manual of Systematics of Archaea and Bacteria, which offers updated descriptions of prokaryotic taxa (Rainey, 2015). Microscopic morphology was observed through a Nikon Eclipse E200 optical microscope from a Gram-stained smear (Madigan *et al*., 2004).

2.4 *Metabolic characterization*

Strains isolated on 0.1% CMC agar plates, with less than 72 hours of growth, were sub-cultured on blood agar, MacConkey, and tryptic soy agar (TSA) and incubated for 24 hours at 37°C to assess their biochemical capacity for hemolysis and lactose hydrolysis. Following this, the strains underwent a series of biochemical tests, including triple sugar iron (TSI), citrate utilization, urea hydrolysis, motility-indole-ornithine (MIO), sulfate reduction-indole-mobility (SIM), nitrate reduction, sucrose, glucose, mannitol, lactose, gelatin liquefaction, hemolysis of bovine erythrocytes, and growth in 7.5% NaCl. Subculture and biochemical tests were carried out in duplicate and the results were interpreted based on Microbiological Diagnosis by Elmer W. Koneman (Koneman, 2012).

2.5 *Determination of cellulolytic activity*

Strains grown on 0.1% CMC agar were re-plated by puncturing onto CMC agar and incubated for 24 hours at 50°C. Congo red dye (1% w/v) was added and left to act for fifteen minutes. Excess dye was then removed and the plates were rinsed with a 0.1 M NaCl solution for 15 minutes. The plates were then left to rest for 24 hours at 4°C and the diameter of the cellulolytic activity ring was measured (Rodríguez & Llenque, 2016). The cellulolytic index (CI) was determined using Equation 1, as reported by Zainudin *et al*. (2022), based on the colony growth diameter and the diameter of the hydrolysis halo. Additionally, the efficiency of carboxymethylcellulose degradation was confirmed through COD measurements.

$$CI = \frac{(Total\ halo\ diameter - Growth\ colony\ diameter)}{Growth\ colony\ diameter} \quad (1)$$

2.6 *Genotypic characterization*

Bacterial DNA from strains with cellulolytic activity was extracted using the Wizard Genomic DNA Purification kit A1120. High-molecular weight DNA (approximately 20 000 bp) was separated by horizontal electrophoresis in 1% agarose gel, stained with SYBR safe (5 μg/mL) and visualized using a molecular weight marker (100 to 3000 bp).

For the amplification and purification of the 16S rRNA gene, a 10^{-1} dilution of the genomic DNA was prepared. PCR amplification was performed according to Aguirre-Garrido *et al*. (2012) using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR products were analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide (5 μg/mL) and compared to a molecular size marker (100 to 3000 bp).

The amplification was confirmed by electrophoresis and the PCR products were subsequently purified using the Wizard SV Gel and PCR Clean-Up kit (Promega; Ref: A9281). The purified DNA was quantified by electrophoresis on a 1% agarose gel. After amplification and purification of the 16S rRNA gene, the samples were sent to MACROGEN Inc. (South Korea) for sequencing.

Once the sequences were obtained, consensus sequences were generated using the BioEdit sequence alignment editor (Hall, 1999). Then, the sequences were compared with the sequences available in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST) program (www.ncbi.

nlm.nih.gov/blast), as described by Aguirre-Garrido et al. (2012). The E value was considered, which is the measure used to estimate the degree of sequence similarity; the closer to 0, the better the alignment and the gaps that refer to the lack of a base in the alignment (Choudhuri, 2014).

The alignment of the sequences was performed using the software ClustalX (Larkin *et al*., 2007). The editing and trimming of the alignments were performed using the program SeaView (Gouy & Gascuel, 2010). Finally, the phylogeny was inferred using the maximum likelihood method and the HKY model (Hasegawa *et al*., 1985). The evolutionary analyses were carried out in MEGA version 12 (Kumar *et al*., 2024).

2.7 Microbial kinetics

Strains that showed halos of cellulose hydrolysis were re-inoculated in serological flasks containing 50 ml of 0.1% CMC broth and incubated at 50°C for 50 hours. For the kinetic study, serological flasks were used as experimental units, to which 60 ml of 0.1% CMC broth were added and inoculated with 10 ml of the previously cultured strain. The experimental units were incubated at 50°C and sampled at different times until reaching growth asymptote. Cell growth was measured indirectly by spectrophotometry (HACH-UV-vis Dr 6000) at a wavelength of 600 nm, using the non-inoculated culture medium as a blank. The microbial growth curve was constructed, the specific growth rate (μ) was determined using the exponential growth model and Gompertz model (Trinidad, 2014), and the doubling time (D_t) was calculated.

3 Results

3.1 Isolation, characterization and identification

The initial compost had a weight of 8.7 kg, a pH of 7.0, a moisture content of 75%, a temperature of 25°C, a COD of 2500 mg/L, and an inorganic nitrogen concentration of 2.5 mg/L. The composting process was successful in all phases and lasted 136 days, yielding 2.7 kg of stable compost. The mesophilic phase lasted 59 days, the thermophilic phase 40 days, the cooling phase 7 days, and the maturation phase 30 days. The results of parameters in the different phases of processing are presented in Table 1. According to the parameters established in the national (NMX-AA-180-SCFI-2018) and international standards such as the Test Methods for the Examination of Composting and Compost [U.S. Department of Agriculture (USDA), 2001] and the composting manual "European Compost Network-Quality Assurance Scheme (ECN-QAS) Manual for Compost and Digestate" [European Compost Network (ECN), 2018], the final compost presented a type 1 quality with a germination rate of 95%.

Among the compost samples in the thermolytic phase, two microbial strains with well-defined morphologies prevailed under the cultivation conditions (i.e., 50°C and CMC medium 0.1%). These strains were labeled as P11 and I12. Strain P11 (Figure 1A) formed punctate colonies with smooth, shiny, raised edges and a dry surface. Strain I12 (Figure 1B) exhibited irregular colonies with convex, filamentous, shiny edges and a dry surface. Regarding the microscopic characterization, both strains were bacillary and Gram positive (Figure 1C-D). Both strains underwent biochemical analysis, the results of which are summarized in Table 2. The results demonstrated the metabolic capability to assimilate glucose, sucrose, and mannitol as carbon sources, produce gas, and reduce nitrates. Additionally, they exhibited motility and growth in 7.5% NaCl (w/v) solution, proteolytic activity on collagen (gelatin), and alpha-hemolysis on bovine erythrocytes.

Based on the biochemical profiles and macro and microscopic morphology, both strains were presumptively identified as belonging to the genus *Bacillus*, following the identification and classification criteria outlined in Bergey's Manual (Vos *et al*., 2011; Koneman, 2012; Mac, 2003). According to the literature, bacteria of the genus *Bacillus* are Gram positive microorganisms capable of forming endospores, allowing them to survive in extreme environments (Beladjal *et al*., 2018). They are facultative aerobes and anaerobes (Harirchi *et al*., 2022) and produce various enzymes, including proteases, amylases, lipases, and cellulases (Yang *et al*., 2021a). Additionally, genus *Bacillus* can metabolize a broad range of carbon sources (e.g., glucose, sucrose, and mannitol) and nitrogen sources (e.g., amino acids, ammonium, nitrate, and nitrite) (Zaprasis et al., 2015).

Table 1. Physicochemical characteristics of the composting phases.

Phase	Phase duration (Day)	Temperature (°C)	pH	Moisture (%)	COD (mg/L)	Inorganic nitrogen (mg/L)
Mesophilic	1-59	29 ± 1	8.56 ± 0.14	67.91 ± 0.42	2450 ± 7.10	2.58 ± 0.20
Thermophilic	59-99	47 ± 2	8.32 ± 0.13	67.80 ± 0.51	1830 ± 14.11	13.58 ± 0.13
Cooling	99-106	30 ± 2	8.21 ± 0.12	80.00 ± 0.50	1013 ± 4.10	12.41 ± 0.21
Maturation	106-136	28 ± 1	7.4 ± 0.09	72.20 ± 0.46	689 ± 5.71	11.43± 0.14

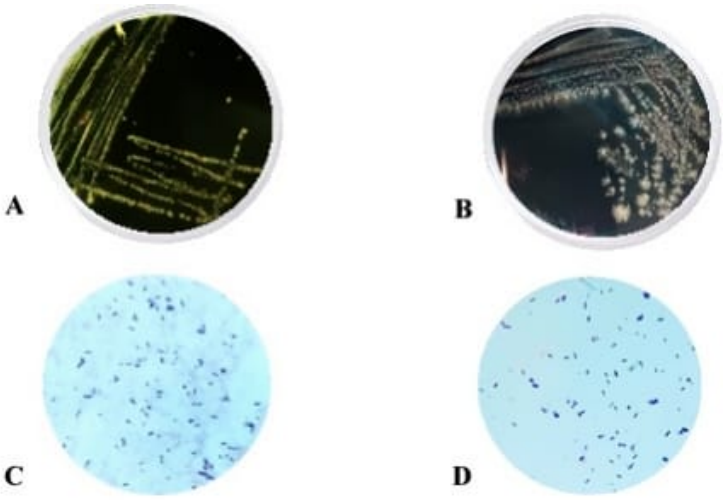

Figure 1. Colonial morphology on CMC 0.1% agar: A) strain P11 and B) strain I12; and Gram stain C) Strain P11 and D) Strain I12.

Table 2. Biochemical test of strains I12 and P11.

Biochemical test	Strain I12	Strain P11
*Glucose, sucrose and lactose	+	+
*Gas production	+	+
*Hydrogen sulfide production	-	-
**Motility	+	+
**Indole	-	-
**Ornitjine	-	-
***Sulfate reduction	-	-
***Indole	-	-
***Motility	+	+
Urea	-	-
Citrate	-	-
Nitrous Broth (nitrate reduction)	+	+
Glucose	+	+
Mannitol	+	+
Sucrose	+	+
Lactose	-	-
Growth in NaCl 7.5%	+	+
Gelatin liquefaction	+	+
Hemolysis of bovine erythrocytes	Alpha hemolysis	Alpha hemolysis

*TSI test, ** MIO test, *** SIM test

The ability of the strains to degrade a wide range of organic substrates suggests their capacity to ferment carbohydrates and adapt to low-oxygen environments by utilizing alternative electron acceptors such as nitrate and fumarate (Rey *et al*., 2004; Blanco *et al* 2024). Motility, likely mediated by flagella-like structures (Ni *et al*., 2024), along with the ability to grow in saline conditions, supports the halotolerant nature of the strains (James *et al*., 2023). Regarding their proteolytic activity on gelatin, this indicates that the bacteria might produce serine proteases and collagenase, which hydrolyze peptide bonds in collagen (Contesini *et al*., 2018; Al-Bedak *et al*., 2023). Additionally, their alpha-hemolytic activity suggests the production of hemolysins, which partially degrade red blood cell membranes (Muras *et al*., 2021).

Identification of strains P11 and I12 at the genus or species level was performed by PCR extraction and amplification of the 16S rRNA gene. For both strains, the Figure 2A shows the DNA extract obtained, with a weight of 20000 bp, and Figure 2B the amplified and purified 16s rRNA gene products a weight of 1500 bp. Sequence analysis in GenBank showed a 97.80% similarity percentage for strain P11 with *Bacillus sp*. and a 100% similarity percentage for strain I12 with *Bacillus licheniformis*. Figure 3 shows the phylogenetic tree where the clustering of *B. sp*. P11 and *B. licheniformis* I12 is shown in bold.

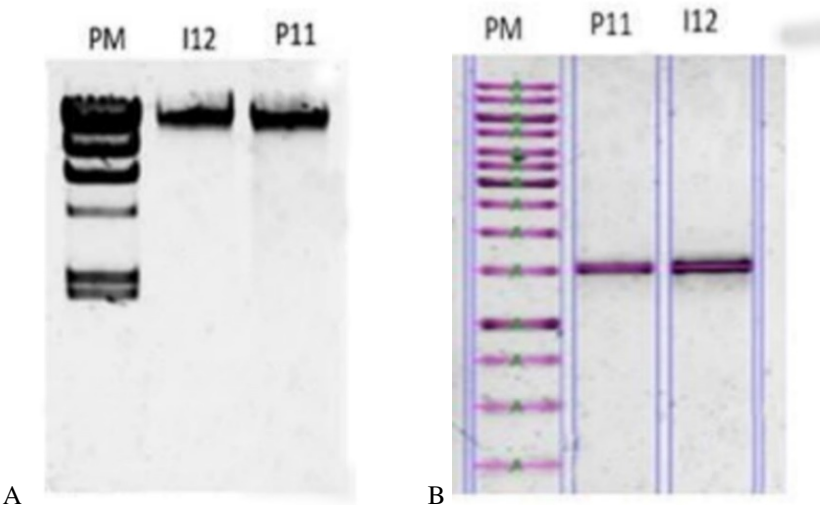

Figure 2. Electrophoresis gel: (A) bacterial DNA extraction, (B) 16s rRNA gene amplification and purification.

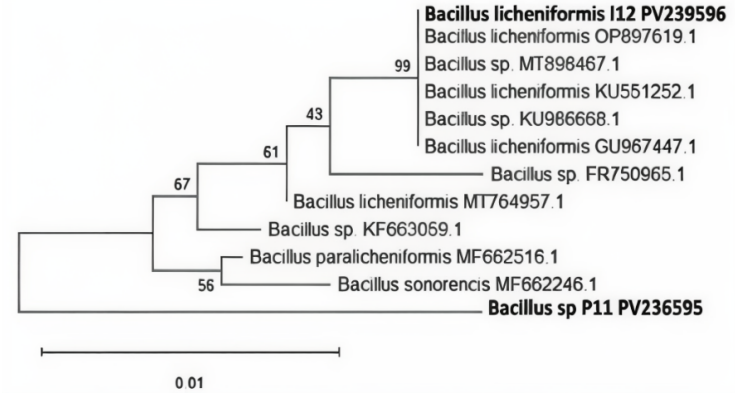

Figure 3. Phylogenetic tree for *Bacillus sp* P11 and *Bacillus licheniformis* I12 inferred by the Maximum Likelihood (ML) method using the nucleotide substitution model of Hasegawa-Kishino-Yano (1985). Twelve sequences with 1,363 positions were analyzed in the final data set. Numbers next to branches represent bootstrap support values, indicating statistical confidence in the clustering of strains within each clade. These values reflect the percentage of replicate trees in which associated taxa clustered (1,000 replicates).

The internal nodes of the phylogenetic tree indicate the proportion of sites where at least one unambiguous base is present in at least one sequence of each descendant clade. The phylogenetic tree suggests that both strains group into well-defined clades with high similarity with other strains within the genus *Bacillus*, although a separation in the tree is observed between the identified strains despite having similar metabolic characteristics. The genus *Bacillus* is highly diverse and includes species with significant genetic variability, even among those sharing similar physiological traits (Xu & Kovács, 2024). Phylogenetic analysis based on the 16S rRNA gene sequence can detect subtle differences that phenotypic tests may overlook, which explains the observed divergence between both genera (Blanco *et al*., 2024)

These results are consistent with the literature where the genus *Bacillus* is reported as the predominant microorganism in the thermophilic phase of composting (López *et al*., 2021; Yang *et al*., 2021b). López *et al*. (2021) identified 159 *Bacillus* and *Firmicutes* strains during composting process of pruning waste and tomato crops, with *Bacillus licheniformis* being one of the most abundant. In the present work, by correlating the molecular and morphological results of the strains, it was possible to corroborate that the characteristics obtained for the *Bacillus licheniformis* strain (I12), such as the lichen-like and cream-coloured colonial morphology, are consistent with the literature. However, it is important to note that the genus *Bacillus* presents a wide range of morphologies, which can vary between species (Logan & Vos, 2015).

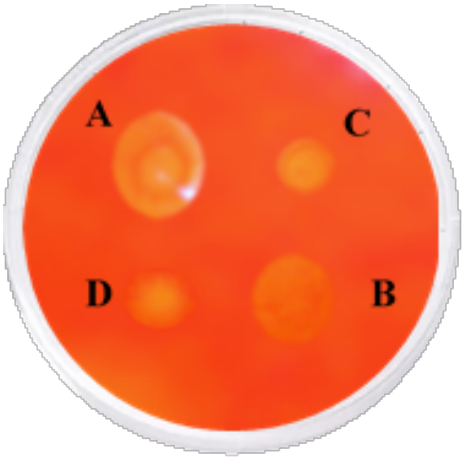

Figure 4. Hydrolysis halos. A and B: *Bacillus sp.,* (P11), C and D: *Bacillus licheniformis* (I12).

3.2 Enzymatic activity and microbial kinetics

Thermophilic microorganisms, such as *Bacillus licheniformis* and *Bacillus sp.,* play a crucial role in composting systems. Through the production of thermostable enzymes, particularly cellulases, they facilitate the efficient degradation of cellulose and other components of plant biomass (Kanekar & Kanekar, 2022; Zhu *et al*., 2020). In the present work, the isolated and characterized strains (i.e., *Bacillus licheniformis* and *Bacillus sp*.) showed their ability to degrade cellulose through a halo formation test using 1% CMC and Congo red agar plates. Both strains showed activity, presenting halos with an average diameter of 10.85 mm ± 0.7 (Figure 4 A-B) and 8 mm ± 0.7 (Figure 2 C-D) for *Bacillus sp.* (P11) and *Bacillus licheniformis* (I12), respectively. The cellulolytic index was determined by calculating the ratio between hydrolysis zone and the colony diameter. The strains *Bacillus sp.* (P11) and *Bacillus licheniformis* (I12) presented CI values of 9.9 ± 0.8 and 7 ± 0.7, respectively. These results highlight the cellulose-degrading ability of the isolated bacteria, which contrasts with the findings of López *et al*. (2021).

López *et al*. (2021) conducted a study during the thermophilic phase of composting (59.4°C ± 6), in which they isolated 159 bacterial strains. The majority belonged to the phylum *Firmicutes* (96%), including *Bacillus licheniformis*, with smaller proportions from *Actinobacteria* (2%) and *Proteobacteria* (2%). However, the isolates exhibited only xylanase activity, with no evidence of cellulolytic activity. Van (2009) documented that *Bacillus licheniformis* presents endoglucanase enzymes that cause the hydrolysis of the glycosidic bonds of cellulose. Medison *et al*. (2023) reported *Bacillus* hydrolysis halos of 5 to 20 mm in a medium with sodium carboxymethylcellulose through the Congo red test on CMC plates at culture temperatures of 28°C. Li *et al*. (2023) reported the isolation of *Bacillus subtilis* from silkworm excrement with a hydrolysis halo diameter of 1.75 mm and CI of 3.5, using sodium carboxymethylcellulose as a carbon source and cultivating it at 55°C with a pH of 6. Vásquez & Millones (2023) reported the isolation from the thermophilic phase of a compost with a temperature of 53.5°C, pH of 6.8 and moisture of 54.8% of *Bacillus subtilis* and *Bacillus safensis subsp. safensis* with a cellulose hydrolysis halo diameter of 7.5 to 8.6 mm and an CI of 0.33 to 0.9, respectively. The variations in hydrolysis halo diameters and cellulolytic index reflect the diversity of the strains and their adaptability to different isolation environments (Hemati et al., 2021; Jiang *et al*., 2021). In this context, the strains isolated in this study are particularly significant, as they exhibited a higher cellulolytic index, highlighting their potential for efficient cellulose degradation.

The CMC degradation efficiency was obtained from the initial and final COD of the strains grown in 0.1% CMC. The degradation percentages obtained for *Bacillus sp* (P11) and *Bacillus licheniformis* (I12) were 0.60% ± 0.01 and 0.49% ± 0.02, respectively. The role of *Bacillus* species in cellulose degradation during the thermophilic phase of composting has been previously reported (Ince *et al*., 2020). In this context, Table 3 presents recalculated cellulose degradation percentages based on enzymatic activity (U) values reported in the literature. These values were estimated using the conversion factor of 1U = 1.8016×10^4 g CMC hydrolyzed, which reflects the amount of substrate degraded per unit of enzyme activity.

Table 3. Comparison based on cellulose degradation percentage.

Reference	Substrate	Degradation (%)	Phase temperature (°C)	Bacteria associated with degradation
This work	CMC 0.1%	0.69 0.49	47	*Bacillus* sp *Bacillus licheniformis*
Ma *et al*. (2020)	*CMC	150 U 0.03**	57.6	*Bacillus*
Liu *et al*. (2020)		11.6 U 0.2**	55	*Bacillus*
Zhang *et al*. (2021)		144 U 2.5**	> 50	*Firmicutes*

U: amount of substrate consumed in μmol/min; *The use of CMC is indicated but not the concentration; **To compare the results obtained in this work, the units were changed to mass units, considering the equivalence of 1U = 1.8016×10^4 g CMC hydrolyzed.

Table 4. Comparison of the kinetic parameters obtained through the Gompertz model and the exponential growth model.

	Bacillus sp. (P11)			*Bacillus licheniformis* (I12)		
Model	μ (h^{-1})	D_t (h)	R^2	μ (h^{-1})	D_t (h)	R^2
Exponential	0.105 ± 0.010	6.5 ± 0.4	0.93	0.420 ± 0.005	1.6 ± 0.5	0.80
Gompertz	0.105 ± 0.005	6.5 ± 0.3	0.99	0.079 h^{-1} ± 0.010	8.8 ± 1.0	0.97

These results confirmed that the strains isolated in this work have a high potential for cellulose degradation. This capacity can be attributed to its enzymatic production and adaptation to composting conditions, reinforcing its applicability in environmental biotechnology, particularly in the treatment and valorization of organic waste through composting (López *et al*., 2021).

On the other hand, Figure 5 shows the microbial growth profiles of both strains, fitted to the Gompertz model. Both strains exhibited a lag phase of approximately 3 hours. Subsequently, *Bacillus sp.* entered the exponential phase, which lasted until hour 30, whereas *Bacillus licheniformis* maintained exponential growth until hour 40. Table 4 shows the specific growth rate (μ) and doubling time obtained using both the Gompertz and exponential growth models. For *Bacillus sp*., both models yielded similar values for growth rate and doubling time. In contrast, for *Bacillus licheniformis*, the Gompertz model provided a better fit to the experimental data, as indicated by a higher correlation coefficient (R^2 = 0.97).

Recent studies on the kinetic parameters of these strains are limited in the literature. Among the limited data available, the D_t varies based on culture conditions. Errington & Aart (2020) reported that *Bacillus* had a D_t of 0.33 hours at 35°C in a nutrient medium. Da Silva *et al*. (2021) reported a doubling time of 6.3 hours at 50°C when using glucose alone or in combination with casein as a carbon source. The influence of culture conditions, particularly the availability of carbon and nitrogen sources, on the growth of thermophilic bacteria is well documented. For instance, Da Silva *et al*. (2021) and O'Hair *et al*. (2020) reported doubling times of 11.5 hours and 0.43 hours, respectively, *for Bacillus licheniformis* grown on CMC and casein. Similarly, Hanlon & Hodges (1981) found that *Bacillus licheniformis* cultured at 37°C with glucose and ammonium chloride had a doubling time of 1 hour. However, when the nitrogen source was replaced with sodium nitrate, alanine, or glutamic acid, the doubling time increased to 1.65, 1.77, and 1.90 hours, respectively, demonstrating that both growth rate and doubling time are highly dependent on substrate availability. Liu *et al*. (2023) further emphasized that culture conditions significantly affect microbial kinetics, with *Bacillus* species maintaining their growth and degradation capacity at high temperatures, though their kinetic behavior varies depending on nutrient availability. Da Silva *et al*. (2021) highlights the importance of composting systems as valuable sources for isolating microorganisms, as the diversity of carbon sources in these environments influences microbial adaptation. In the case of lignocellulosic waste, bacteria degrade complex compounds such as cellulose, leading to shorter doubling times under similar culture conditions, particularly when using CMC as a carbon source (Zhu *et al*., 2021).

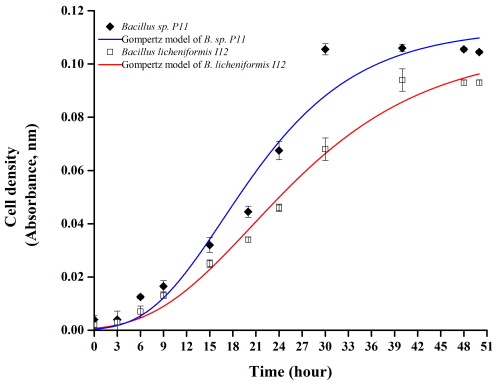

Figure 5. Cell growth curve and Gompertz model fitting of strain *B. sp* P11 and *B. licheniformis* I12.

Understanding key kinetic parameters, such as the specific growth rate and doubling time, provides deeper insights into microbial metabolism on different substrates. This knowledge enables better control of fermentation processes, the optimization of bioreactor design, and the development of strategies to enhance the production of valuable enzymes (Straathof, 2023). Currently, research is focused on exploring the applications of these bacterial enzymes in the biodegradation of lignocellulosic waste to obtain nanocellulose (Herrera-Basurto *et al*., 2024) and the production of biofuels or biomaterials such as polyhydroxyalkanoates (Castilla-Marroquín *et al*., 2024)

Conclusions

The two thermophilic bacterial strains isolated from the thermophilic phase of an urban organic waste compost were identified through macro and microscopic, metabolic, and genetic analyses as *Bacillus sp*. and *Bacillus licheniformis*. Their kinetic characterization

revealed growth rates and doubling times of 0.105 h^{-1} ± 0.005; 6.5 h ± 0.3 and 0.0794 h^{-1} ± 0.01; 8.8 h ± 1, respectively. Both strains exhibited significant cellulolytic activity, with hydrolysis halos of 10.85 mm ± 0.7 and 8 mm ± 0.7, and cellulolytic indexes of 9.9 ± 0.8 and 7 ± 0.7, respectively. These findings highlight the critical role of composting systems as reservoirs of thermostable cellulolytic microorganisms with valuable biotechnological applications. Furthermore, the metabolic and kinetic characterization of these strains provides a strong foundation for future research aimed at optimizing composting processes and enhancing the bioconversion of lignocellulosic waste into valuable products. Their potential application in sustainable waste management and bioresource recovery underscores their relevance in environmental biotechnology.

Acknowledgements

This work was part of a project financed by Secretaría de Investigación y Estudios Avanzados (SIEA) UAEMéx, México, code number: 7276/2025CIB.

References

Aguirre-Garrido, J. F., Montiel-Lugo, D., Hernández-Rodríguez, C., Torrescortes, G., Millán, V., Toro, N., Martínez-Abarca, F. and Ramírez-Saad, H. C. (2012). Bacterial community structure in the rhizosphere of three cactus species from semiarid highlands in central Mexico. *Antonie van Leeuwenhoek* 101, 891-904. https://doi.org/10.1007/s10482-012-9705-3

Al-Bedak, O. A.-H. M., Moharram, A. M., Hussein, N. A.-G., Taha, D. M., Stephenson, S. L., and Ameen, F. (2023). Microbial Exploitation of Feather Wastes for Sustainable Production of Keratinase and Collagenase Enzymes by Didymella keratinophila AUMC 15399 in Submerged Fermentation. *Fermentation*, 9(6), 507. https://doi.org/10.3390/fermentation9060507

Anguiano Aguilar, E. (2019). Aislamiento, selección y caracterización de bacterias con actividad celulolítica. Tesis de maestría, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, México.

Babu, R., Veramendi, P. M. P. and Rene, E. R. (2021). Strategies for resource recovery from the organic fraction of municipal solid waste. *Case studies in chemical and environmental engineering 3*, 100098. https://doi.org/10.1016/j.cscee.2021.100098

Banerjee, A., Cornejo, J. and Bandopadhyay, R. (2020). Emergent climate change impact throughout the world: call for "Microbiome Conservation" before it's too late. *Biodiversity and Conservation* 29, 345-348. https://doi.org/10.1007/s10531-019-01886-6

Beladjal, L., Gheysens, T., Clegg, J. S., Amar, M. and Mertens, J. (2018). Life from the ashes: survival of dry bacterial spores after very high temperature exposure. *Extremophiles* 22, 751–759. https://doi.org/10.1007/s00792-018-1035-6

Blanco Crivelli, X., Cundon, C., Bonino, M. P., Sanin, M. S. and Bentancor, A. (2024). The Complex and Changing Genus Bacillus: A Diverse Bacterial Powerhouse for Many Applications. *Bacteria*, 3(3), 256-270. https://doi.org/10.3390/bacteria3030017

Cao, X., Williams, P. N., Zhan, Y., Coughlin, S. A., McGrath, J. W., Chin, J. P. and Xu, Y. (2023). Municipal solid waste compost: Global trends and biogeochemical cycling. *Soil & Environmental Health 1*, 100038. https://doi.org/10.1016/j.seh.2023.100038

Castilla-Marroquín, J. D., Pacheco, N., Herrera-Corredor, J. A., Hernández-Rosas, F., Jiménez-Morales, K., Benítez-Salamanca, M. J., and Hernández-Martínez, R. (2024). Polyhydroxyalkanoates Production by Bacillus thuringiensis HA1 Using Sugarcane Molasses as Carbon Source. *Revista Mexicana de Ingeniería Química* 23(3), 1-12. https://doi.org/10.24275/rmiq/Bio24352

Choudhuri, S. (2014). Sequence Alignment and Similarity Searching in Genomic Databases: BLAST and FASTA. *Bioinformatics for Beginners*, (Academic Press), Pp. 133-155. https://doi.org/10.1016/B978-0-12-410471-6.00006-2

Contesini, F. J., Melo, R. R. D. and Sato, H. H. (2018). An overview of Bacillus proteases: from production to application. *Critical reviews in biotechnology* 38, 321-334. https://doi.org/10.1080/07388551.2017.1354354

Da Silva, R. N., de Andrade Melo, L. F. and Finkler, C. L. L. (2021). Optimization of the cultivation conditions of *Bacillus licheniformis* BCLLNF-01 for cellulase production. *Biotechnology Reports*, 29. https://doi.org/10.1016/j.btre.2021.e00599

De Mendonça Costa, L. A., de Mendonça Costa, M. S. S., Damaceno, F. M., Chiarelotto,

M., Bofinger, J. and Gazzola, W. (2021). Bioaugmentation as a strategy to improve the compost quality in the composting process of agro-industrial wastes. *Environmental Technology & Innovation 22*, https://doi.org/10.1016/j.eti.2021.101478

Environmental Protection Agency. (2021). Standards for the Use or Disposal of Sewage Sludge https://www.federalregister.gov/documents/2001/12/21/01-31342/standards-for-the-use-or-disposal-of-sewage-sludge

Errington, J. and Aart, L. T. V. D. (2020). Microbe Profile: *Bacillus subtilis*: model organism for cellular development, and industrial workhorse. *Microbiology* 166, 425-427. https://doi.org/10.1099/mic.0.000922

European Compost Network. ECN – QAS Manual for Compost and Digestate. (2018) https://www.compostnetwork.info/ecn-qas/ecn-qas-manual/

Firone I., Feola A., Russo L., Cattaneo, A., Di Donato P., Nicolaus B. and Romano I. (2023). Thermophilic bacteria and their thermozymes in composting processes: a review. *Chemical and Biological Technologies in Agriculture 10*, 7. https://doi.org/10.1186/s40538-023-00381-z

Getahun, T., Nigusie, A., Entele, T., Van Gerven, T., and Van der Bruggen, B. (2012). Effect of turning frequencies on composting biodegradable municipal solid waste quality. *Resources, Conservation and Recycling 65*, 79-84. https://doi.org/10.1016/j.resconrec.2012.05.007

Gouy M., Guindon S., and Gascuel O. (2010) SeaView version 4 : a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution 27(2):221-224. https://doi.org/10.1093/molbev/msp259

Guo, R., Li, G., Jiang, T., Schuchardt, F., Chen, T., Zhao, Y., and Shen, Y. (2012). Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. *Bioresource technology 112*, 171-178. https://doi.org/10.1016/j.biortech.2012.02.099

Hall, T. A. (1999, January). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *In Nucleic acids symposium series* 41, 95-98.

Hanlon, G. W. and Hodges, N. A. (1981). Bacitracin and protease production in relation to sporulation during exponential growth of *Bacillus licheniformis* on poorly utilized carbon and nitrogen sources. *Journal of bacteriology* 147, 427–431. https://doi.org/10.1128/jb.147.2.427-431.1981

Harirchi, S., Sar, T., Ramezani, M., Aliyu, H., Etemadifar, Z., Nojoumi, S. A., Yazdian, F., Awasthi, M. K. and Taherzadeh, M. J. (2022). Bacillales: From Taxonomy to Biotechnological and Industrial Perspectives. *Microorganisms* 10, 2355. https://doi.org/10.3390/microorganisms10122355

Hasegawa, M., Kishino, H., and Yano, T. (1985). Datinf the human-ape split by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22, 160-174.

Hemati, A., Aliasgharzad, N., Khakvar, R., Khoshmanzar, E., Lajayer, B. A. and van Hullebusch, E. D. (2021). Role of lignin and thermophilic lignocellulolytic bacteria in the evolution of humification indices and enzymatic activities during compost production. *Waste Management*, 119, 122-134. https://doi.org/10.1016/j.wasman.2020.09.042

Herrera-Basurto, R., Ramos-López, E., Rodríguez-López, A., González-Olvera, J. C., Morales-Hernández, J., Hurtado-Macías, A., Vergara-Hernández, H. J., and Mercader-Trejo, F. (2024). Study on wild grasses as a potential source of cellulose nanofibers. *Revista Mexicana de Ingeniería Química* 23(3), 1-12. https://doi.org/10.24275/rmiq/Mat24245

Hussian, C. H. A. C., and Leong, W. Y. (2023). Thermostable enzyme research advances: a bibliometric analysis. *Journal of Genetic Engineering and Biotechnology* 21, 37. https://doi.org/10.1186/s43141-023-00494-w

Ince, O., Ozbayram, E. G., Akyol, Ç, Erdem, E. I, Gunel, G., and Ince, B. (2020). Bacterial succession in the thermophilic phase of composting of anaerobic digestates. *Waste and Biomass Valorization* 11, 841–849. https://doi.org/10.1007/s12649-018-0531-3

James, N., Umesh, M., Sarojini, S., Shanmugam, S., Nasif, O., Alharbi, S. A., Chi, L.T.N. and Brindhadevi, K. (2023). Unravelling the potential plant growth activity of halotolerant Bacillus licheniformis NJ04 isolated from soil and its possible use as a green bioinoculant on Solanum lycopersicum L. *Environmental Research*, *216*, 114620. https://doi.org/10.1016/j.envres.2022.114620

Jiang, J., Wang, Y., Yu, D., Yao, X., Han, J., Cheng, R., Cui, H., Yan, G., Zhang, X. and Zhu G. (2021). Garbage enzymes effectively regulated the succession of enzymatic activities and the bacterial community during sewage sludge composting. Bioresour Technology 327, 1–10. https://doi.org/10.1016/j. biort ech.2021.124792

Kanekar, P. P., and Kanekar, S. P. (2022). Thermophilic, thermotolerant microorganisms. *In Diversity and Biotechnology of Extremophilic Microorganisms from India* , Pp.117-153. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-1573-4_4

Koneman, E., Winn Jr, W., Allen, S., Janda, W., Procop, G., Schreckenberber, P. and Woods, G. (2012). Diagnóstico microbiológico: texto e atlas colorido. In *Diagnóstico microbiológico*: texto e atlas colorido (pp. xxxv-1565).

Kumar, S,. Stecher, G,. Suleski, M,. Sanderford, M,. Sharma, S., and Tamura, K. (2024). Molecular Evolutionary Genetics Analysis Version 12 for adaptive and green computing. *Molecular Biology and Evolution* 41, 1-9. https://doi.org/10.1093/molbev/msae263

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947-2948. https://doi.org/10.1093/bioinformatics/btm404

Li, H., Zhang, M., Zhang, Y., Xu, X., Zhao, Y., Jiang, X., Zhang, R., and Gui, Z. (2023). Chracterization of Cellulose-Degrading Bacteria Isolated from Silkworm Excrement and Optimization of Its Cellulase Production. *Polymers* 15, 4142. https://doi.org/10.3390/polym15204142

Li, J., Sun, L., and Huo, Y. X. (2025). High-Temperature Catalytic Platform Powered by Thermophilic Microorganisms and Thermozymes. *Synthetic Biology and Engineering*, 3(1), 10001. https://doi.org/10.70322/sbe.2025.10001

Liu, G., Zhang, K., Gong, H., Yang, K., Wang, X., Zhou, G., Cui, W., Chen, Y., and Yang, Y. (2023). Whole genome sequencing and the lignocellulose degradation potential of Bacillus subtilis RLI2019 isolated from the intestine of termites. *Biotechnol Biofuels* 16, 130. https://doi.org/10.1186/s13068-023-02375-3

Liu, H., Huang, Y., Wang, H., Shen, Z., Qiao, C., Li, R. and Shen, Q. (2020). Enzymatic activities triggered by the succession of microbiota steered fiber degradation and humification during co-composting of chicken manure and rice husk. *Journal of environmental management* 258, 110014. https://doi.org/10.1016/j.jenvman.2019.110014

Logan, N. A. and Vos, P. D. (2015). Bacillus. *Bergey's manual of systematics of archaea and bacteria*, Pp. 1-163.

López, M. J., Jurado, M. M., López-González, J. A., Estrella-González, M. J., Martínez-Gallardo, M. R., Toribio, A., and Suárez-Estrella, F. (2021). Characterization of thermophilic lignocellulolytic microorganisms in composting. *Frontiers in microbiology* 12, 697480. https://doi.org/10.3389/fmicb.2021.697480

Lynd, L., Weimer, P., Van Zyl, W. H. and Pretorius, I. S. (2022). Microbial Cellulose Utilization: Fundamentals and Biotechnology. *Microbiology and molecular biology reviews* 3, 506–577. https://doi.org/10.1128/mmbr.66.3.506-577.2002

Ma, C., Jin, H. J., Hu, B., Liu, N., Zhang, K., Zhao, J. H. and Zhang, H. Z. (2020). Changes in Enzyme Activity and Bacterial Succession During Sewage Sludge Composting. *Nature Environment & Pollution Technology* 19, 695-701. https://doi.org/10.46488/NEPT.2020.v19i02.024

Mac Faddin, J. (2003). Pruebas bioquimicas para la Identificacion de bacterias de Importancia Clinica. 3era Edicion. Editorial Médica Panamericana. Buenos Aires-Argentina.

Madigan, M. T., Martinko, J. M. and Parker, J. (2004). Brock. Biología de los microorganismos.

Medison, R. G., Jiang, J., Medison, M. B., Tan, L. T., Kayange, C. D., Sun, Z. and Zhou, Y. (2023). Evaluating the potential of *Bacillus licheniformis* YZCUO202005 isolated from lichens in maize growth promotion and biocontrol. *Heliyon 9* https://doi.org/10.1016/j.heliyon.2023.e20204

Moreno, J., López-González, J. A., Arcos-Nievas, M. A., Suárez-Estrella, F., Jurado, M. M., Estrella-González, M. J. and López, M. J. (2021). Revisiting the succession of microbial populations throughout composting: a matter of thermotolerance. Science of The Total Environment 773,145587. https://doi.org/10.1016/j.scitotenv.2021.145587

Muras, A., Romero, M., Mayer, C. and Otero, A. (2021). Biotechnological applications of *Bacillus licheniformis*. *Critical Reviews in Biotechnology* 41, 609-627. https://doi.org/10.1080/07388551.2021.1873239

Nguyen, V. T., Le, T. H., Bui, X. T., Nguyen, T. N., Vo, T. D. H., Lin, C., Vu, T. M. H., Nguyen, H. H., Nguyen, D. D., Senoro D. B. and Dang, B. T. (2020). Effects of C/N ratios and turning frequencies on the composting process of food waste and dry leaves. *Bioresource Technology Reports*, *11*, 100527. https://doi.org/10.1016/j.biteb.2020.100527

Ni, S., Wu, Y., Zhu, N., Leng, F. and Wang, Y. (2024). Bacillus licheniformis YB06: A Rhizosphere–Genome-Wide Analysis and Plant Growth-Promoting Analysis of a Plant Growth-Promoting Rhizobacterium Isolated from Codonopsis pilosula. *Microorganisms*, *12*(9), 1861. https://doi.org/10.3390/microorganisms12091861

O'Hair, J., Jin, Q., Yu, D., Poe, N., Li, H., Thapa, S., Zhou, S. and Huang, H. (2020). Thermophilic and alkaliphilic *Bacillus licheniformis* YNP5-TSU as an ideal candidate for 2, 3-butanediol production. *ACS Sustainable Chemistry & Engineering* 8, 11244-11252. https://doi.org/10.1021/acssuschemeng.0c02759

Rainey, F., Kämpfer, P., Trujillo, M., Chun, J., DeVos, P., Hedlund, B. and Dedysh, S. (2015). Bergey's manual of systematics of Archaea and Bacteria (Vol. 410). W. B. Whitman (Ed.). Hoboken, NJ.

Rey, M. W., Ramaiya, P., Nelson, B. A., Brody-Karpin, S. D., Zaretsky, E. J., Tang, M., Lopez de Leon, A., Xiang, H., Gusti, V., Clausen, I. G., Olsen, P. B., Rasmussen, M. D., Andersen, J. T., Jorgenses, P. L., Larsen, T. S., Sorokin, A., Bolotin, A., Lapidus, A., Galleron, N., Ehrlich, S. D., and Berka, R. M. (2004). Complete genome sequence of the industrial bacterium Bacillus licheniformis and comparisons with closely related *Bacillus* species. *Genome biology 5*, 1-12. https://doi.org/10.1186/gb-2004-5-10-r77

Rodríguez Silva, L. A., y Llenque Díaz, L. (2016). Aislamiento y selección de bacterias celulolíticas a partir de compost de residuos orgánicos. *REBIOL* 36, 19-28. https://revistas.unitru.edu.pe/index.php/facccbiol/article/view/1310

Secretaría de Economía. (2018). NMX-AA-180-SCFI-2018: Que establece los métodos y procedimientos para el tratamiento aerobio de la fracción orgánica de los residuos sólidos urbanos y de manejo especial, así como la información comercial y de sus parámetros de calidad de los productos finales. Diario Oficial de la Federación. https://dof.gob.mx/nota_detalle.php?codigo=5539165&fecha=26/09/2018#gsc.tab=0

Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). (1985). NMX-AA-52-1985. Protección al ambiente- contaminación del suelo-residuos sólidos municipales-preparación de muestras en el laboratorio para su análisis. Diario Oficial de la Federación. http://legismex.mty.itesm.mx/normas/aa/aa052.pdf

Siu-Rodas, Y., de los Angeles Calixto-Romo, M., Guillén-Navarro, K., Sánchez, J. E., Zamora-Briseno, J. A. and Amaya-Delgado, L. (2018). *Bacillus subtilis* with endocellulase and exocellulase activities isolated in the thermophilic phase from composting with coffee residues. *Revista Argentina de Microbiología 50*, 234-243. https://doi.org/10.1016/j.ram.2017.08.005

Straathof, A. J. (2023). Modelling of end-product inhibition in fermentation. *Biochemical Engineering Journal* 191, 108796. https://doi.org/10.1016/j.bej.2022.108796

Trinidad Bello, A. (2014). Modelos de crecimiento en biología, su significado biológico y selección del modelo por su ajuste [Tesis de licenciatura, Universidad Autónoma Metropolitana - Iztapalapa]. Repositorio Institucional UAM. https://bindani.izt.uam.mx/concern/tesiuams/dj52w481t

Van Dyk, J. S. (2009). Characterization of the Cellulolytic and Hemicellulolytic System of *Bacillus Licheniformis SVD1* and the Isolation and Characterization of a Multi-enzyme Complex. Tesis de Doctorado, Rhodes University.

Vásquez, E. and Millones, C. (2023). Isolation and Identification of Bacteria of Genus Bacillus from Composting Urban Solid Waste and Palm Forest in Northern Peru. *Microorganisms*, 11, 751. https://doi.org/10.3390/microorganisms11030751

Vos, P., Garrity, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., ... & Whitman, W. B. (Eds.). (2011). Bergey's manual of systematic bacteriology: Volume 3: The Firmicutes (Vol. 3). Springer Science & Business Media.

Wang, C., & Kuzyakov, Y. (2024). Mechanisms and implications of bacterial-fungal competition

for soil resources. *The ISME journal*, 18(1), wrae073. https://doi.org/10.1093/ismejo/wrae073

Waqas, M., Hashim, S., Humphries, U. W., Ahmad, S., Noor, R., Shoaib, M., Naseem, A., Hlaing P. T. and Lin, H. A. (2023). Composting processes for agricultural waste management: a comprehensive review. *Processes 11*, 731. https://doi.org/10.3390/pr11030731

Xu, X., & Kovács, Á. T. (2024). How to identify and quantify the members of the Bacillus genus?. *Environmental Microbiology*, 26(2), e16593. https://doi.org/10.1111/1462-2920.16593

Yang, H., Qu, J., Zou, W., Shen, W. and Chen, X. (2021a). An overview and future prospects of recombinant protein production in *Bacillus subtilis*. *Applied Microbiology and Biotechnology* 105, 6607-6626. https://doi.org/10.1007/s00253-021-11533-2

Yang, J., Zhao, J., Jiang, J., Xu, H., Zhang, N., Xie, J. and Wei, M. (2021b). Isolation and characterization of *Bacillus sp*. capable of degradating alkali lignin. *Frontiers in Energy Research* 9, 807286. https://doi.org/10.3389/fenrg.2021.807286

Zainudin, M. H. M., Singam, J. T., Sazili, A. Q., Shirai, Y. and Hassan, M. A. (2022). Indigenous cellulolytic aerobic and facultative anaerobic bacterial community enhanced the composting of rice straw and chicken manure with biochar addition. Scientific Reports, 12(1), 5930. https://doi.org/10.1038/s41598-022-09789-3

Zaprasis, A., Bleisteiner, M., Kerres, A., Hoffmann, T. and Bremer, E. (2015). Uptake of amino acids and their metabolic conversion into the compatible solute proline confers osmoprotection to *Bacillus subtilis*. *Applied and Environmental Microbiology 81*, 250-259. https://doi.org/10.1128/AEM.02797-14

Zhang, S., Wang, J., Chen, X., Gui, J., Sun, Y. and Wu D. (2021). Industrial-scale food waste composting: effects of aeration frequencies on oxygen consumption, enzymatic activities, and bacterial community succession. *Bioresource Technology* 320, 1–10. https://doi.org/10.1016/j.biortech.2020.124357

Zhu, D., Adebisi, W. A., Ahmad, F., Sethupathy, S., Danso, B. and Sun, J. (2020). Recent development of extremophilic bacteria and their application in biorefinery. *Frontiers in Bioengineering and Biotechnology* 8, 483. https://doi.org/10.3389/fbioe.2020.00483

Zhu, N., Gao, J., Liang, D., Zhu, Y., Li, B,. and Jin, H. (2021). Thermal pretreatment enhances the degradation and humification of lignocellulose by stimulating thermophilic bacteria during dairy manure composting. *Bioresource Technology* 319, 1–9. https://doi.org/10.1016/j.biortech.2020.124149