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Prospection of microbial enzymes for plastic biodegradation from plastic dumpsite in Tepic, Nayarit, Mexico.

Prospección de enzimas microbianas para la biodegradación de plásticos de un tiradero de plásticos en Tepic, Nayarit.

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ABSTRACT

The environmental impact of plastics has promoted the search for biotechnological strategies for their degradation. This study isolated and characterized microorganisms with hydrolytic and oxidoreductase enzymatic activity from a contaminated site called “Arena Cora” in Nayarit, Mexico. 23 plastic waste samples were collected, isolating 172 strains. A preliminary screening of halo formation in a solid medium at 30 °C for 48h was performed to identify extracellular enzymes, followed by quantification through submerged fermentation at 30 °C, 150 rpm for 72h, both with inducers. Microorganisms were identified using MALDI-TOF Biotyper (MALDI-TOF-MS mass spectrometry) to identify the proteomic signature. The enzymatic screening in solid medium identified 22 strains with relevant activity, with *Bacillus* (76.4%) and *Pseudomonas* (11.8%) being the most prevalent genera. The most frequent activities were esterase/lipase and hydrolase, with 48 and 34 positive strains, respectively. 6.4% of these strains reached the highest of both activity levels. Furthermore, five strains with cutinase activity and 15 with laccase activity were identified. Submerged fermentation confirmed the production of hydrolytic and oxidoreductase extracellular enzymes. *B. subtilis* (A55A) exhibited the highest induced activity with tributyrin and mineral oil, reaching over 120 U/mL at 24h. *B. cereus* (A41C) showed the highest cutinase activity (17.05 U/mL at 72h). The highest laccase production was observed in strain A46E (10.3 U/mL at 72h). *P. koreensis* (A58F), with no prior records of plastic degradation, displayed lipase activity at 24h. These results highlight the potential of native microorganisms in plastic biodegradation and their potential application in bioremediation strategies.

KEY WORDS: Pollution, plastics, prospection, microorganisms, extracellular enzymes, biodegradation.

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RESUMEN

El impacto ambiental de los plásticos ha impulsado la búsqueda de estrategias biotecnológicas para su degradación. En este estudio se aislaron y caracterizaron microorganismos con actividad enzimática hidrolítica y oxidoreductasa de un sitio contaminado en Nayarit, México, denominado “Arena Cora”. Se recolectaron 23 muestras de residuos plásticos, aislando 172 cepas. Se realizó un primer cribado por halos en medio sólido a 30 °C por 48h para identificar enzimas extracelulares, seguido de una cuantificación por fermentación sumergida a 30 °C, 150 rpm por 72h, ambos con inductores. La identificación de microorganismos se realizó por MALDI Biotyper (espectrometría de masas MALDI-TOF-MS) para identificar las firmas proteómicas. El cribado enzimático en medio sólido identificó 22 cepas con actividad relevante, destacando los géneros *Bacillus* (76.4 %) y *Pseudomonas* (11.8 %). Las actividades más frecuentes fueron esterasa/lipasa e hidrolasa, con 48 y 34 cepas positivas, respectivamente. Un 6.4 % de las cepas alcanzó el nivel más alto de ambas actividades. Además, se identificaron cinco cepas con actividad cutinasa y 15 con lacasa. La fermentación sumergida confirmó la producción de enzimas hidrolíticas y oxidoreductasas extracelulares. *B. subtilis* (A55A) tuvo la mayor actividad inducida con tributirina y aceite mineral, alcanzando más de 120 U/mL a las 24h. *B. cereus* (A41C) presentó la mayor actividad cutinasa (17.05 U/mL a las 72 h). Para lacasas, la mayor producción se obtuvo en la cepa A46E (10.3 U/mL a las 72 h). *P. koreensis* (A58F), sin antecedentes en la degradación de plásticos, mostró actividad lipasa a las 24h. Estos resultados evidencian el potencial de microorganismos nativos en la biodegradación de plásticos y su posible aplicación en biorremediación.

PALABRAS CLAVE: Contaminación, plásticos, prospección, microorganismos, enzimas extracelulares, biodegradación.

Introduction

The extensive use of plastics has increased exponentially since the second half of the 20th century (Geyer *et al.,* 2017). Due to their malleability and versatility, plastics have multiple applications, ranging from the food sector to the pharmaceutical industry. It is estimated that global plastic production exceeds 460 million tons per year (Ritchie *et al.,* 2023), of which only 9% is recycled, exacerbating waste accumulation and its environmental impact (Griffin & Karaski, 2022).

Approximately eight million tons of plastic reach the oceans each year (Stanley *et al.,* 2025), largely due to inadequate waste management, industrial discharges, and insufficient

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collection and disposal systems in coastal areas. These plastics act as pollutants, often carrying pesticides, detergents, and industrial chemicals, among others, which affect marine life and pose risks to human health when ingested (Landrigan *et al.,* 2020). Furthermore, the fragmentation process generates microplastics (<5 mm) (Acosta González *et al.,* 2022) and nanoplastics (<1000 nm) (Morillo-Velarde Martínez, 2021), which have been detected even in bottled water and marine organism tissues.

Currently, more than a hundred types of plastics have been identified, traditionally grouped into seven main categories. Among the most widely used are polyethylene terephthalate (PET), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyvinyl chloride (PVC), polystyrene (PS), and polypropylene (PP). Given the scarcity of efficient methods for managing and disposing of these polymers, new alternatives for their degradation and potential reuse are required.

In this context, several studies highlight the ability of bacteria to hydrolyze and mineralize plastic polymers through specialized enzymatic pathways. *Ideonella sakaiensis* 201-F6 can degrade PET films in approximately six weeks through the action of two enzymes, PETase and MHETase (Yoshida *et al.,* 2016). These findings demonstrate the potential of enzymatic bioremediation at an industrial scale, although significant challenges remain regarding enzyme optimization, process scalability, and adaptation to variable environmental conditions (Dhali *et al.,* 2024).

Other enzymes, such as hydrolases, esterases (EC 3.1.1.1), lipases (EC 3.1.1.3), and cutinases (EC 3.1.1.74), have shown effectiveness in breaking ester bonds, generating monomers or simpler fragments that can be assimilated by microorganisms (Rueda Rueda *et al.,* 2020; Charnock, 2021). Additionally, laccases (EC 1.10.3.2) can oxidize the polymeric bonds of plastics containing aromatic, oxygenated, or unsaturated groups, generating reactive species that, in turn, break down long polymer chains, facilitating their degradation into smaller fragments (Kumar *et al.,* 2016; Brugnari *et al.,* 2021).

The search for microorganisms in areas with high levels of plastic waste enables the isolation of strains with enzymatic activities relevant to degradation. Bhardwaj *et al.* (2012) proposed a biodegradation mechanism in two stages: enzyme adhesion to the polymeric substrate, followed by hydrolysis to form simpler compounds. Another model, described in five steps, includes microbial adhesion, biodeterioration, biofragmentation, assimilation, and mineralization (Méndez, 2024). Regions with severe plastic pollution provide an ideal setting for isolating native bacteria capable of secreting hydrolytic enzymes of interest. Agroindustrial activities, tourism, and poor waste management contribute to plastic waste accumulation, leading to areas with high concentrations of solid plastics.

The objective of this study is to isolate microorganisms from plastic-contaminated sites in an illegal dumping ground in Tepic, Nayarit. This research focuses on determining the ability of microorganisms to secrete hydrolase- and oxidoreductase-type enzymes associated with plastic degradation. The identification and characterization of such strains could contribute to the development of environmental remediation strategies and the implementation of sustainable waste management methods, both locally and globally.

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Material and Methods

Reagents

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), alpha-cyano-4-hydroxycinnamic acid (HCCA), sodium acetate, acetic acid, mineral oil, formic acid, trichloroacetic acid (TCA), potassium caseinate, fructose, glycerol, glycine, p-nitrophenyl butyrate (p-NPB), p-nitrophenol (p-NP), tributyrin, ninhydrin, Tween 80, monobasic potassium phosphate, potassium iodate, pentahydrated dibasic sodium phosphate, sodium phosphate, sodium chloride, and monobasic sodium phosphate were purchased from Sigma Aldrich (St. Louis, MO, USA) with a purity greater than 98%. Extra virgin linseed oil was obtained from a local supermarket. Nutrient agar, bacteriological agar, yeast extract, casein peptone, and glucose were acquired from MCD LAB (Tultitlán, Edomex, MX).

Culture media

Nutrient agar (AN: 23 g/L nutrient agar, 5 g/L bacteriological agar), YPD agar (20 g/L bacteriological agar, 10 g/L yeast extract, 20 g/L casein peptone, 20 g/L dextrose), nutrient medium (10 g/L yeast extract, 20 g/L casein peptone), and YPD medium (10 g/L yeast extract, 20 g/L casein peptone, 20 g/L dextrose).

Sampling site and sample collection

The site known as “Arena Cora” is an illegal dumping ground located in the Camichín de Jauja community, Tepic, Nayarit, Mexico. Its coordinates are 21°29′33.558″ N and 104°47′35.07″ W. The site has been in use for eight years and is located near the industrial zone (Figure 1). A total of 23 samples were collected using a randomized block design to ensure representative coverage. A 10-meter-long by 2-meter-wide area was divided into five 2 m^2 blocks, defined based on a rectangular geographic layout. Within each block, 4-5 samples with evident signs of abiotic degradation were randomly selected, and spaced one meter apart to minimize sampling bias. Each sample included small fragments of plastic materials, including PS, PP, PET, PUR, LDPE, and HDPE, all showing visible degradation. The samples were placed in labeled plastic bags and stored at 4 °C until analysis.

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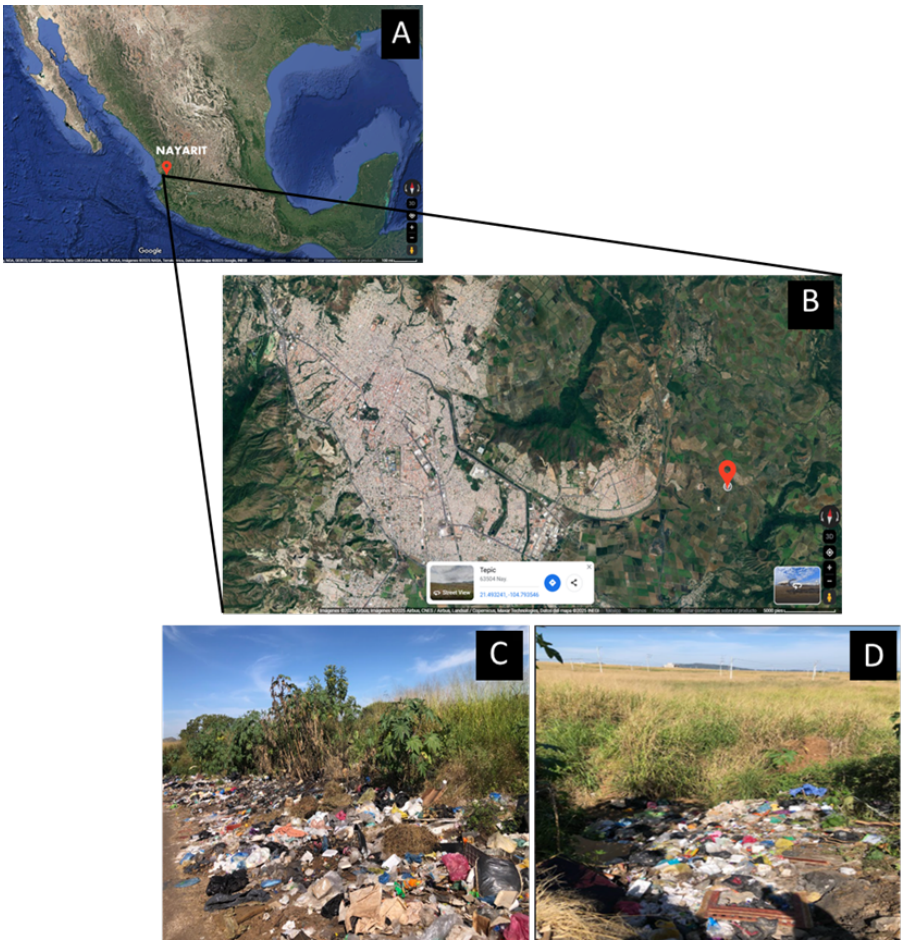

Figure 1. Clandestine dumpsite in Tepic, Nayarit, Mexico.

A) Aerial image of the location of Tepic City retrieved from Google Maps. B) Image of the location of the clandestine dump taken from Google Maps. C-D) Image of the area with plastics in an advanced state of degradation.

Isolation of microorganisms

Three grams of sample, consisting of 2 g of plastic and 1 g of sediment, were suspended in 30 mL of sterile distilled water. Serial dilutions from 10^{-10} to 10^{-25} were prepared for each suspension. Then, 100 µL of each dilution was spread on AN and YPD agar and incubated for 24 h at 30 °C. Colonies with distinct morphologies were streaked onto the original medium using the cross-streak method until pure strains were obtained.

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Enzymatic screening on solid medium

The isolated strains were evaluated for four enzymatic activities using different inducers: esterase/lipase, mineral oil hydrolases, cutinase, and laccase. (1) Esterase/lipase medium (g/L): tryptone 10, yeast extract 5, bacteriological agar 20, tributyrin emulsion (TB) 10. (2) Mineral oil hydrolase medium (g/L): tryptone 10, yeast extract 5, bacteriological agar 20, mineral oil emulsion (MO) 10. (3) Cutinase medium (g/L): $NaNO_3$ 3, K_2HPO_4 1, KCl 0.5, $FeSO_4 \cdot 7H_2O$ 0.01, bacteriological agar 20, linseed oil emulsion (LO) 15. (4) Laccase medium (g/L): nutrient agar 23, bacteriological agar 5, ABTS 1 mM. The media were sterilized by autoclaving at 121 °C for 15 min. The emulsions, ABTS, and powdered milk were added to the corresponding media before plating and stored at 4 °C until use. A total of 172 strains were inoculated and incubated on the five media at 30 °C, with monitoring at 24, 48, and 72 h. A scoring system was established to evaluate the strains in solid-medium screening as follows: Level 0: no growth. Level 1: low growth, no halo. Level 2: high growth, no halo. Level 3: moderate growth with a small halo (<1 mm). Level 4: high growth with a medium halo (>1 mm, <3 mm). Level 5: high growth with a large halo (>3 mm). Strains classified as Levels 4 and 5, which expressed extracellular enzymes associated with plastic degradation, were identified by MALDI-TOF. Those classified as Level 5 were further evaluated for enzyme production in a liquid medium.

Identification by MALDI-TOF

The identification of isolated microorganisms was performed using mass spectrometry with the Bruker Daltonik MALDI Biotyper system (Bremen, Germany). Fresh biomass from isolated colonies was placed in duplicate onto a 96-well stainless steel plate, covered with 1 µL of formic acid (70% v/v), and 1 µL of a saturated HCCA matrix solution, allowing it to dry between additions. The analysis was performed using the Flexcontrol v3.4 software with the MBT_FC.par method conditions.

Enzyme production in liquid medium

Strains with the largest halos in the solid-medium screenings were selected for extracellular enzyme production in a liquid medium. A 30-mL culture of each strain was prepared in nutrient medium or YPD medium in a 250-mL Erlenmeyer flask, incubated at 30 °C for 24 h at 150 rpm. Optical density at 600 nm (Synergy HT Biotek, 270223) was measured, and the required volume was collected to start a 30-mL culture at an OD_{600} of 0.6. The calculated volume was centrifuged to discard the supernatant and resuspended in 5 mL of the corresponding medium (esterase/lipase, cutinase, polyesterase, laccase, or protease) to generate the inoculum. Fermentations were carried out in 125-mL baffled flasks with 25 mL of medium containing the inducer and 5 mL of inoculum. Cultures were incubated at 150 rpm for 72 h at 30 °C. Growth and enzymatic activity were quantified every 24 h over three days by taking 1 mL samples.

For carboxylesterase production, a minimal medium (MM) was prepared with the following composition (g/L): $MnSO_4 \cdot H_2O$ 0.001, KCl 0.5, $NaNO_3$ 3, yeast extract 0.5, $MgSO_4 \cdot 7H_2O$ 0.5,

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K_2HPO_4 1, $FeSO_4 \cdot 7H_2O$ 0.01, $(NH_4)2SO_4$ 1, tryptone 20, dextrose 5, $CuSO_4 \cdot 7H_2O$ 0.0001, and 10 g/L inducer emulsion. For esterase/lipase, mineral oil hydrolase, and cutinase production, the inducers were tributyrin, mineral oil, and linseed oil, respectively, added at the time of inoculation as an emulsion (200 g/L, 1% v/v Tween 80). For laccase production, nutrient medium (8 g/L) and $CuSO_4 \cdot 5H_2O$ (2 mM) were used as inducers.

Bacterial growth

A 350-µL aliquot of the culture was transferred to a 96-well microplate, and absorbance at 600 nm was measured. The medium containing the emulsion was used as the blank.

Enzymatic activity

Enzyme extracts were obtained by centrifuging each sample at 10,000 g for 2 min at 24 °C and stored at 4 °C. Each measurement was performed in triplicate, and results are presented as mean ± standard deviation.

Carboxylesterase activity. Carboxylesterase activity was assessed for cultures induced with tributyrin, mineral oil, and linseed oil, using p-nitrophenyl butyrate (p-NPB) as a substrate, adapting the method reported by Casas-Godoy *et al.* (2023). A reaction mixture containing 20 µL of enzyme extract, 125 µL of saline buffer (NaH_2PO_4, NaCl 100 mM, pH 7.2), and 5 µL of p-NPB (40 mM) in 2M2B was prepared. The reaction was monitored at 37 °C for 20 min, with readings taken every 30 s at 405 nm. One unit of enzymatic activity was defined as the amount of enzyme required to release 1 µmol of p-NP per minute under the tested conditions.

Laccase activity. Laccase activity quantification was based on the work of Kumar *et al.* (2016) with modifications. A reaction mixture containing 20 µL of enzyme extract and 180 µL of 1 mM ABTS solution in 0.1 M acetate buffer (pH 4.5) was prepared. The reaction was monitored at 30 °C for 15 min at 436 nm. Activity calculations used an extinction coefficient of $\epsilon = 2.9 \times 10^4$ cm^{-1} M^{-1} (Kumar *et al.,* 2016). One unit of enzymatic activity was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per minute under the experimental conditions.

Results and Discussion

Isolation of cultivable species from the Arena Cora site

A site is considered contaminated when the presence of substances reaches levels that affect human health, fauna, and the environment (De Jesus & Ruiz, 2012). At the “Arena Cora” site (Tepic), synthetic polymer accumulation has been observed, leading to changes in the diversity and abundance of bacteria, fungi, and yeasts compared to non-contaminated environments. The biodegradation of plastics such as PET, HDPE, LDPE, PS, and PP occurs through extracellular enzymes with hydrolytic activity on their surfaces (Mohanan *et al.,* 2020). Various environmental factors may enhance microbial activity, enabling the isolation and study of microorganisms with

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potential plastic-degrading capabilities.

Among the 23 collected samples, seven were from PET, five from LDPE, four were from PS, one was from PP, and six consisted of a combination of plastic types. The distribution of these plastics in the site, primarily single-use containers, corresponds to the types commonly used for human consumption in metropolitan areas. The isolation of bacteria on non-selective media such as AN and YPD allowed for morphological variability among cultivable colonies in both media. A total of 172 strains were isolated and used for enzymatic screening on a solid medium, where positive strains formed clearance halos or changes in coloration.

This screening aimed to evaluate the ability of microorganisms to produce enzymes with potential plastic-degrading activity. Specifically, carboxylesterase activity was assessed using tributyrin, mineral oil, and linseed oil as inducers. Additionally, laccase activity was evaluated using ABTS. The scoring criteria, as previously described, are presented in Figure 2.

The screening results indicate that esterase/lipase activities and mineral oil hydrolases had the highest number of positive strains, with a total of 48 (27.9%) and 34 (19.8%), respectively. However, only 6.4% of these strains, for both inducers, were classified as level 5, meaning they exhibited high growth and a large degradation halo. Other inducers that have been considered in the search for plastic-degrading enzymatic activities include PET and PCL (Danso *et al.,* 2019).

For cutinases, five positive strains were identified, of which only two (2.91%) were classified as level 5. In previous studies, during a cutinase screening using linseed oil, only 4.75% of 400 strains tested positive (Adiguzel & Tuncer, 2017). Finally, for laccases, 15 positive strains were identified, three of which were classified as level 5.

Identification through MALDI-TOF

Based on the enzymatic screening, 22 strains classified as level 5 for at least one enzymatic activity were selected for identification using MALDI-TOF. The analysis identified 17 of these strains, all of which were bacteria, with 76.4% belonging to the *Bacillus* genus and 11.8% to the *Pseudomonas* genus (Table 1).

Among the identified strains, *Bacillus cereus* (A39H, A46B, A57C, and A58A) and *Bacillus subtilis* (A55A) exhibited high enzymatic activity in the presence of tributyrin and mineral oil as inducers. *B. cereus* has been reported to degrade various polymers, including PE, PET, PS, HDPE, and LDPE (Gambarini *et al.,* 2022). The degradation of HDPE by this species has been confirmed through weight loss monitoring assays (Gupta *et al.,* 2023). Additionally, its ability to degrade PET, PS, and PE microplastics has been documented using infrared spectroscopy and scanning electron microscopy (SEM) (Auta *et al.,* 2017). *B. cereus* has also been isolated from LDPE samples, with an associated degradation rate of 35.7 ± 4.0% for this polymer (Muhonja *et al.,* 2018).

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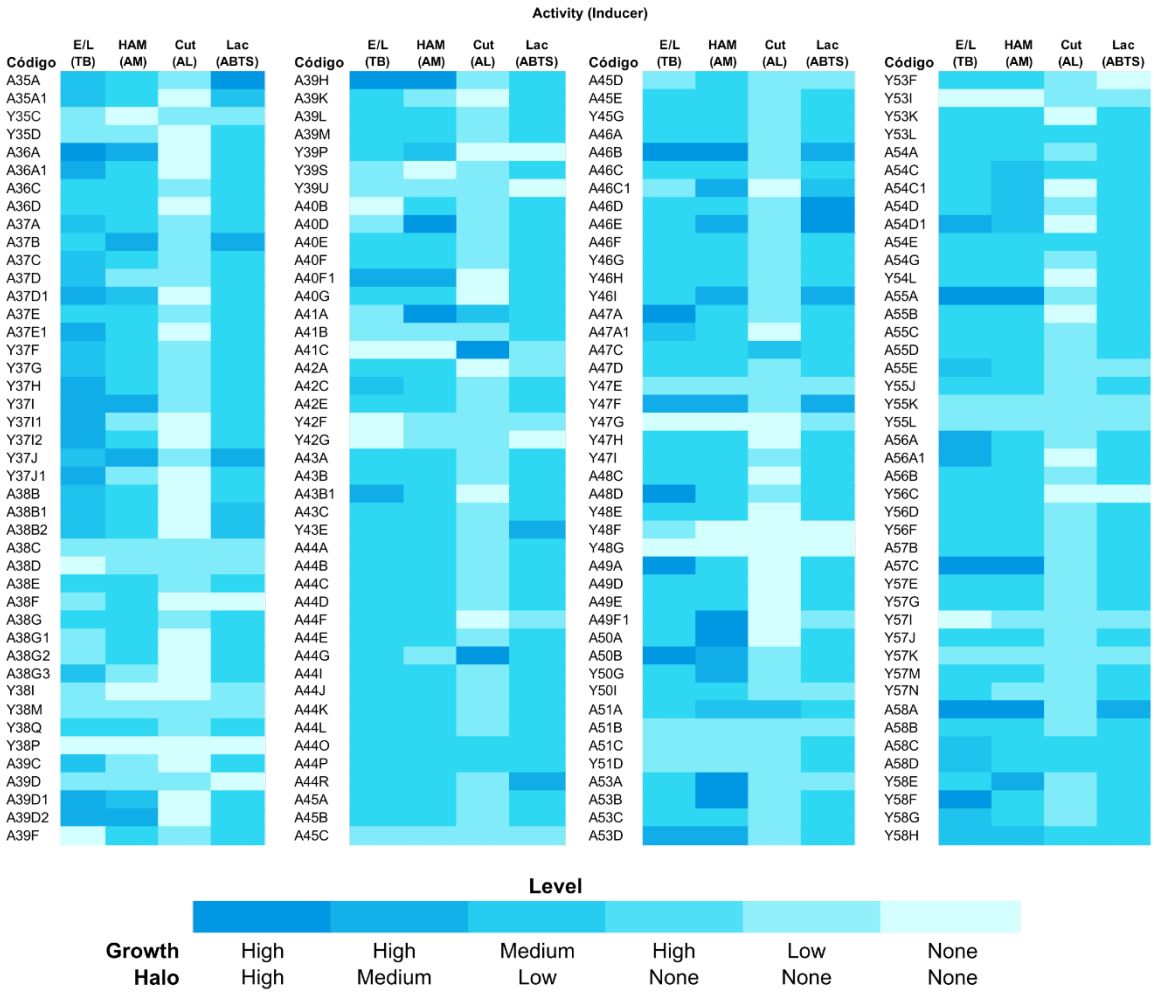

Figure 2. Enzymatic screening of strains isolated from the "Arena Cora" site.

E/L: esterase/lipase, tributyrin inducer (TB); MOH: mineral oil hydrolases, mineral oil inducer (MO); CUT: cutinases, linseed oil inducer (LO); Lac: laccases, ABTS inducer.

B. subtilis has been shown to efficiently degrade HDPE. In a study conducted in India using samples from a coastal area, its degradative potential was demonstrated through weight loss, viability assays, and infrared spectroscopy (Sangeetha Devi *et al.,* 2019). Furthermore, in combination with *Bacillus flexus*, it achieved a 1.5% reduction in the weight of UV-pretreated PP (Aravinthan *et al.,* 2016). Additionally, *B. subtilis* degraded 16.1% of LDPE strips over four months (Pathak & Navneet, 2023), while *Bacillus thuringiensis* has been shown to cause surface damage to PE (Yun *et al.,* 2023).

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Within the *Pseudomonas* genus, *Pseudomonas chlororaphis* is the only identified species with reported polymer degradation capabilities. Its potential was evaluated by cloning and expressing two genes encoding intracellular lipases in *Escherichia coli*. The enzymatic activity was assessed using suspensions of polyhydroxyalkanoate (PHA) polymers, demonstrating its ability to hydrolyze PLA, PHA, PES, and PCL (Mohanan *et al.,* 2022).

Table 1. Strains identified through MALDI-TOF.

Code	Microorganism	Code	Microorganism
A35A	*Pseudomonas taiwanensis*	A48D	*Bacillus cereus*
A36A	*Bacillus cereus*	A49A	Unidentified
A39H	*Bacillus cereus*	A49F	Unidentified
A40D	*Bacillus amyloliquefaciens ssp. plantarum*	A50A	*Glutamicibacter mysorens*
A41A	Unidentified	A50B	*Bacillus thuringiensis*
A41C	*Bacillus cereus*	A53A	*Bacillus marisflavi*
A44G	*Klebsiella oxytoca*	A53B	Unidentified
A46B	*Bacillus cereus*	A55A	*Bacillus subtilis*
A46D	*Pseudomonas chlororaphis*	A57C	*Bacillus cereus*
A46E	Unidentified	A58A	*Bacillus cereus*
A47A	*Bacillus cereus*	Y58F	*Bacillus subtilis*

Additionally, in a study by Howard *et al*. (1999), *P. chlororaphis* was cultured in a medium containing Impranil (aliphatic polyester polyurethane) as the sole carbon source to determine its ability to degrade polyurethane (PU). A clearing of the culture medium was observed, and enzymatic activity of secreted proteins was detected during the degradation process (Howard *et al.,* 1999).

Enzymatic activity via submerged fermentation

Induction with tributyrin

Esterases and lipases are hydrolases capable of breaking carboxyl esters, allowing them to participate in the degradation of various polyesters (Tchigvintsev *et al.,* 2015). The production of these enzymes was evaluated in 11 strains, induced with tributyrin, and quantified every 24 h over a period of 72 h (Table 2). The highest activity was observed in strains A55A (127.0 ± 2.4 U/mL) at 24 h and A58A (51.8 ± 5.4 U/mL) at 72 h.

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Figure 3 presents the activity kinetics for A55A and A58A following induction with tributyrin, along with growth estimation by turbidity at OD_{600nm}. Figure 3a illustrates that *B. subtilis* (A55A) exhibited a stationary phase with sustained enzymatic expression between 24 and 72 h, reaching peak esterase/lipase activity at 24 h. In contrast, Figure 3b shows that *B. cereus* (A58A) displayed a progressive increase in enzymatic expression from 24 to 72 h, with maximum growth at 24 h. Species from the *Bacillus* genus have been extensively associated with the degradation of various polyester plastics. Both *B. cereus* (Auta *et al.,* 2017) and *B. subtilis* (Maheswaran *et al.,* 2023) have been studied for their role in PET degradation.

Table 2. Evaluation of esterase/lipase enzymatic activity.

Strain	Bacteria	Enzymatic activity (U/mL)
A36A	*Bacillus cereus*	$15.3^{a} \pm 6.7$
A39H	*Bacillus cereus*	$10.8^{c} \pm 1.9$
A46B	*Bacillus cereus*	$13.1^{c} \pm 1.5$
A47A	*Bacillus cereus*	$37.2^{a} \pm 2.2$
A48D	*Bacillus cereus*	$30.7^{b} \pm 2.3$
A49A	Unidentified	$11.9^{b} \pm 4.8$
A50B	*Bacillus thuringiensis*	$14.6^{c} \pm 4.4$
A55A	*Bacillus subtilis*	$127.0^{a} \pm 2.4$
A57C	*Bacillus cereus*	$7.6^{c} \pm 3.1$
A58A	*Bacillus cereus*	$51.8^{c} \pm 5.4$
Y58F	*Pseudomonas koreensis*	$27.6^{a} \pm 6.5$

Maximum enzyme activity quantified at 24 h (a); 48 h (b); 72 h (c).

Is *P. koreensis* particularly interesting, as there are currently no reports linking it to plastic degradation or esterase activity. However, Anbu (2014) reported extracellular lipase activity in this species, with a preference for medium- and long-chain fatty acids TG (10:0-18:0). In this study, *P. koreensis* exhibited sustained activity and growth up to 72h, with peak expression at 24 h when induced with tributyrin (Figure 3c), making it a promising candidate for polyester plastic degradation.

Induction with mineral oil

Mineral oil, composed of crude oil-derived alkanes, was used as an inducer to assess the production of hydrocarbon-degrading enzymes. The induction was evaluated in 11 strains, with enzymatic activity quantified every 24 h over 72 h (Table 3). The highest enzymatic activities were observed at 24 h in strain A55A (*B. subtilis*) with 129.8 U/mL, followed by strain A41A (unidentified) with 85.9 U/mL.

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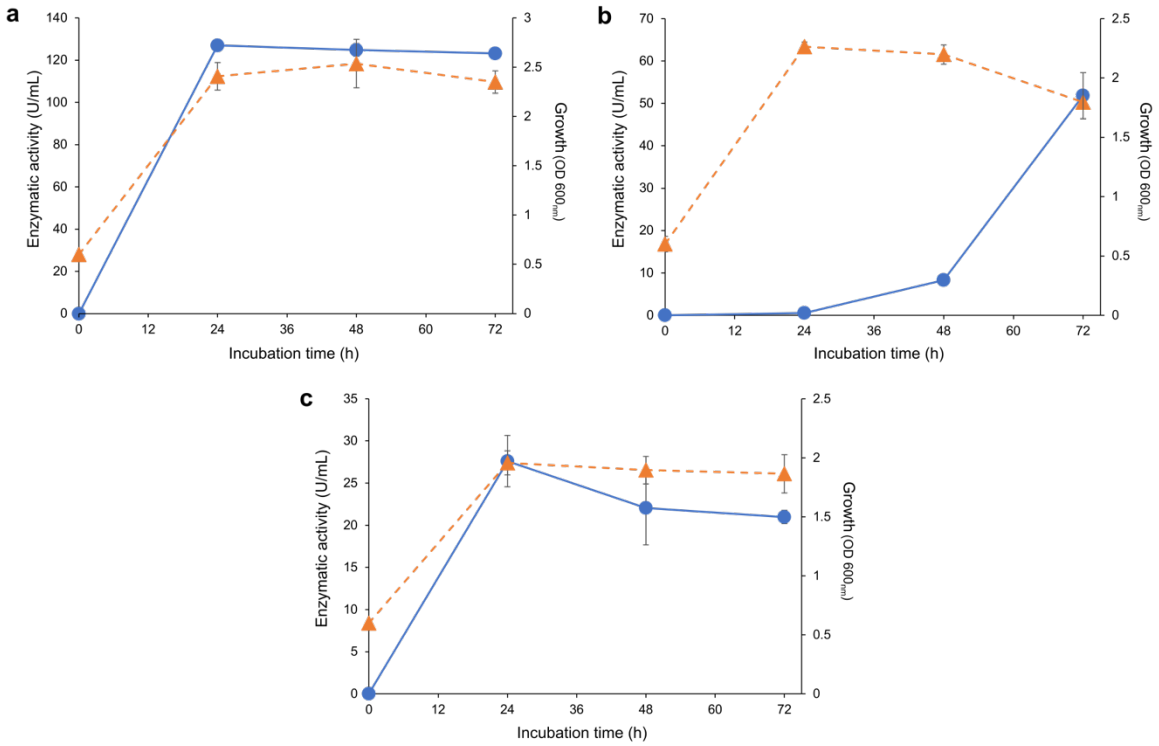

Figure 3. Kinetics of esterase/lipase activity (●) and growth (▲) of bacteria induced with tributyrin.

a) *B. subtilis* (55A), b) *B. cereus* (58A), c) *P. koreensis* (58F).

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Table 3. Evaluation of enzymatic activity induced with mineral oil.

Strain	Bacteria	Enzymatic activity (U/mL)
A39H	*Bacillus cereus*	$14.2^{c} \pm 5.4$
A40D	*Bacillus amyloliquefaciens spp. plantarum*	$27.7^{a} \pm 2.6$
A41A	Unidentified	$85.9^{a} \pm 9.3$
A46B	*Bacillus cereus*	$7.8^{a} \pm 0.6$
A49F	Unidentified	$22.5^{c} \pm 6.9$
A50B	*Bacillus thuringiensis*	$13.6^{c} \pm 3.5$
A53A	*Bacillus marisflavis*	$14.4^{c} \pm 4.3$
A53B	Unidentified	$12.0^{c} \pm 6.0$
A55A	*Bacillus subtilis*	$129.8^{a} \pm 3.6$
A57C	*Bacillus cereus*	$6.5^{c} \pm 3.3$
A58A	*Bacillus cereus*	$23.7^{c} \pm 1.7$

Maximum enzyme activity quantified at 24 h (a); 48 h (b); 72 h (c).

The enzymatic expression of *B. subtilis* in the presence of mineral oil (Figure 4a) showed a 61% reduction after reaching its peak at 24h, coinciding with its transition to the stationary growth phase. *B. subtilis* has been demonstrated to efficiently degrade both saturated and unsaturated hydrocarbons through the production of alkane hydrolases, enzymes that cleave hydrocarbon bonds (Darsa *et al.,* 2014; Parthipan *et al.,* 2017).

Meanwhile, Figure 4b depicts strain A41A, whose growth appears correlated with its enzymatic expression. In addition to *B. subtilis,* several bacteria have been reported to degrade petroleum-derived hydrocarbons, including *Pseudomonas aeruginosa, Alcanivorax borkumensis, Rhodococcus erythropolis, Achromobacter xylosoxidans*, and *Burkholderia cepacia.* These species have demonstrated the ability to produce a variety of enzymes, such as oxidases, dehydrogenases, oxygenases, and alkane hydrolases (Kadri *et al.,* 2018; Lin *et al.,* 2024). The study of enzymatic expression in the presence of low-molecular-weight alkanes provides a rapid method for assessing plastic degradation potential.

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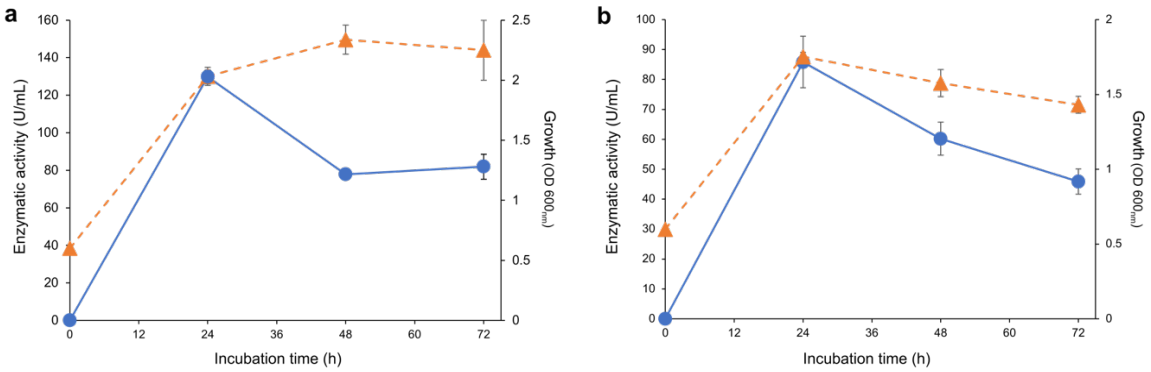

Figure 4. Kinetics of hydrolytic activity (●) and growth (▲) of bacteria induced with mineral oil for the strains a) *B. subtilis* (A55A), b) Unidentified (A41A).

Induction with linseed oil

Cutinase activity has been linked to plastic degradation due to its structural and catalytic similarities with other hydrolases, such as PETases, lipases, and esterases (Retnadhas *et al.,* 2024). These enzymes have been isolated from fungi, bacteria, and plants. In this study, linseed oil was used to induce cutinase production. From the four positive strains identified in solid medium, the two-level 5 strains (Figure 2) were selected to evaluate their extracellular enzymatic production. Both strains exhibited activity starting at 48h; however, *B. cereus* (A41C) reached its peak activity (17.05 U/mL) at 72 h (Table 4).

Table 4. Evaluation of enzymatic activity induced with linseed oil.

Strain	Bacteria	Enzymatic activity (U/mL)
A41C	*Bacillus cereus*	$17.1^{c} \pm 3.2$
A44G	*Klebsiella oxytoca*	$4.2^{b} \pm 4.1$

Maximum enzyme activity quantified at 24 h (a); 48 h (b); 72 h (c).

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The *Bacillus* genus has been reported to produce cutinases, with activities of 15 U/mL over four days using apple cutin (Adiguzel & Tuncer, 2017). Meanwhile, *Klebsiella oxycota* has only been associated with hydrocarbon degradation, such as petroleum (Rabelo Florez & Márquez Gómez, 2022).

Laccase activity

Laccases are polyphenol oxidase enzymes that contain copper ions in their structure, allowing them to catalyze the oxidation of a wide range of phenolic substrates and aromatic amines, reducing molecular oxygen to water (Lin *et al.,* 2023). These enzymes have been extensively studied and isolated from various biological sources, including plants, insects, bacteria, and mainly fungi.

Cruz Pérez (2016) proposed a metabolic pathway in which certain fungi utilize laccase and lignin peroxidase for LDPE degradation, employing mediators and inducers. This mechanism begins with ABTS oxidation by laccase, followed by manganese peroxidase activity, which uses cupric ions and hydrogen peroxide to weaken the polymer bonds. Finally, lignin peroxidase acts to convert the molecule into oligomers.

From the 15 positive strains in solid medium screening for laccase activity (Figure 5), three were evaluated in submerged fermentation for 72 h (Table 5). The highest enzymatic production was observed in strain A46E at 72h, reaching 10.3 U/mL. Reports on bacterial laccases are scarce; however, a cloned and expressed *Acinetobacter baumannii* laccase in *E. coli* produces 0.159 U/mL (Zhang *et al.,* 2022), a lower enzymatic activity compared to strain A46E. Despite being positive in solid medium, strain A46D showed low extracellular activity, while no activity was detected in strain A35A.

Table 5. Evaluation of laccase enzymatic activity.

Strain	Bacteria	Enzymatic activity (U/mL)
A35A	*Pseudomonas taiwanensis*	NC
A46D	*Pseudomonas chlororaphis*	$0.2^{c} \pm 0.06$
A46E	Unidentified	$10.3^{c} \pm 0.28$

Maximum enzyme activity quantified at 24 h (a); 48 h (b); 72 h (c).

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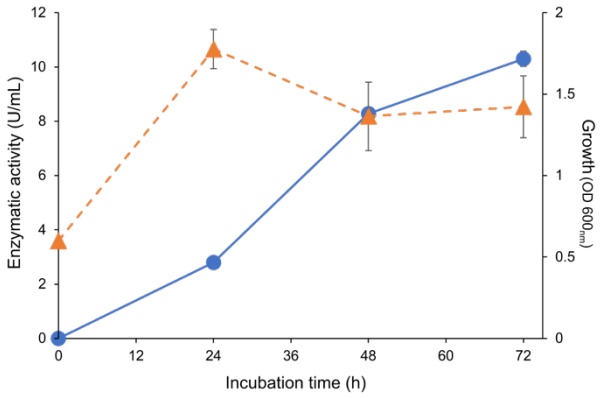

Figure 5. Kinetic of laccase activity (•) and growth (▲) for A46E bacteria.

Conclusions

This study enabled the isolation and characterization of microorganisms with potential for plastic degradation from a highly contaminated site in Tepic, Nayarit, Mexico. A total of 172 strains were identified, with 22 exhibiting significant enzymatic activity, highlighting species from the *Bacillus* and *Pseudomonas* genera. Enzymatic screening in solid medium revealed that esterase/lipase and mineral oil hydrolase activities were the most prevalent among the isolated strains. Regarding cutinases and laccases, only a small percentage exhibited high activity.

Submerged fermentation analysis confirmed the production of hydrolytic and oxidoreductase enzymes with potential for polyester plastic degradation. *B. cereus* and *B. subtilis* demonstrated significant esterase and cutinase production, while *P. koreensis*, previously unreported in plastic degradation, exhibited lipase activity induced by tributyrin, suggesting its potential role in polymer degradation.

The results confirm the importance of exploring contaminated environments for microorganism identification. The strain with the highest enzymatic activity, *B. subtilis* (A55A), is a promising candidate for developing biotechnological strategies in plastic waste management. However, further studies are required to characterize the enzymatic mechanisms at the molecular level and assess their efficiency under large-scale degradation conditions.

Author contributions

Work conceptualization, LCG, MRM; methodology development, YAL; software management, LCG, MK, and ACV; experimental validation, MTSM, MRM; data analysis, YAL,

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ASG, and MRM; data management, YAL, MRM; manuscript writing and preparation, LCG, YAL, ASG, and MRM; manuscript review and editing, LCG, ASH, and MRM; project administration, LCG, MRM; funding acquisition, LCG, MRM.

All authors have read and approved the published version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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