RESEARCH ARTICLE

PET-degrading microorganisms isolated from residues of the SAV biosphere reserve and identification of carboxylic ester hydrolase activity during their growth in the presence of polymer

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Abstract: The "Sistema Arrecifal Veracruzano" (SAV) is a vital marine ecosystem; its resources are continually perturbed due to contamination by anthropogenic activities, with multiple contaminants such as plastics. In aquatic ecosystems, plastics are almost immediately coated by inorganic and organic matter, which is then colonized by microbes to form a biofilm on plastic surfaces. This work aimed to isolate and identify plastic-degrading microorganisms isolated mainly from plastic residues of the SAV biosphere reserve. Eight bacteria and three fungi were isolated from a biofilm in plastic residues from islands of SAV. All the bacteria and one fungus showed evidence of degrading PET, over 10% for two bacteria and 17% for the fungus. All fungi belong to the genus Aspergillus, and bacteria belong to the genera Aneurinibacillus, Bordetella, Bacillus, and Lysinibacillus. *Aneurinibacillus migulanus* and *Aspergillus flavus* showed the highest values for PET degradation. Carboxylic ester hydrolase (CEH) activity was detected in all crude extracts from fungi and bacteria growing with PET triturates as a carbon source; the maximum CEH in bacteria was 255 U mg^{-1} and 780 U mg^{-1} for fungi.

Keywords: The Sistema Arrecifal Veracruzano, plastic degradation, Aneurinibacillus, Aspergillus, carboxylic ester hydrolase (CEH)

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Received: January 15, 2024; **Accepted:** April 20, 2024; **Published Online:** April 28, 2024

Citation: Narciso-Ortiz, L., Peña-Montes, C., Peralta-Peláez, L.A., Fernández-Romero, F., Anzures-Jacinto, M.L., Sulbarán-Rangel, B., Vázquez-Alcántara, L.C., Córdoba-Silveira, S.N., Pacheco-Rivera, K.M., 2024. PET-degrading microorganisms isolated from residues of the SAV biosphere reserve and identification of carboxylic ester hydrolase activity during their growth in the presence of polymer. *Applied Environmental Biotechnology*, 9(1): 11-21. http://doi.org/10.26789/AEB.2024.01.002

1 Introduction

The "Sistema Arrecifal Veracruzano" (SAV) is a coral reef ecosystem located in the southern Gulf of Mexico, on the continental shelf of Veracruz state, off the coast of Boca del Río, Alvarado municipalities (Dussud et al., 2018). The SAV is a Biosphere Reserve (UNESCO, 2006), a National Marine Park since 1992 (DOF, 1992), and Ramsar site number 1346 (SISR, 2004). The SAV is a vital ecosystem due to its ability to protect the coast, reduce the intensity of extreme hydrometeorological events, and provide economic activities such as tourism and maritime trade (Escamilla-Pérez et al., 2021). SAV resources are continually perturbed due to contamination by anthropogenic activities, i.e., marine traffic, wastewater and agriculture discharges, cities and industrial development, tourism activities and the expansion project of Port of Veracruz (Mapel-Hernández et al., 2021; Ramírez-Macas et al., 2018). Anthropogenic activities signify the presence of multiple contaminants in the SAV, such as heavy metals (Montoya-Mendoza et al., 2019), pesticides (Martinez et al., 2017), hydrocarbons, or plastics (Narciso-Ortiz et al., 2020). Plastics are now indispensable for several uses, such as electronics, construction, engineering, packaging, healthcare, and textiles, due to their low cost, durability, and strength (Amobonye et al., 2020; Zhao et al., 2022). Increasing plastic production frequently leads to the entry of its residues into aquatic ecosystems as the SAV, representing an immense environmental problem with negative consequences for ecosystems because plastic residues take many years to be degraded in the environment (Danso et al., 2019). Some microorganisms produce enzymes that catalyze plastic polymers degradation; the most famous is the bacterium *Ideonella sakaiensis*, which degrades polyethylene terephthalate (PET) and the intermediate mono(2-hydroxyethyl)

terephthalic acid (MHET) in terephthalic acid and ethylene glycol by two enzymes: PETase and MHETase (Yoshida et al., 2021). In fungi organisms, cutinase enzymes are associated with PET degradation; for example, the cutinase enzyme MRCUT1 of *Moniliophthora roreri*, when used for heterologous expression in *Escherichia coli*, degraded 31% of PET (Vázquez-Alcántara et al., 2021). The first stage of biodegradation is biodeterioration, which starts with the adherence of microorganisms on the plastic surface (Amobonye et al., 2020; Asiandu et al., 2020). In aquatic ecosystems, plastics are almost immediately coated by inorganic and organic matter, which is then colonized by microbes to form a biofilm on the plastic surface (Dussud et al., 2018). Microbial biofilms that form on plastic residues are named "plastisphere" and can develop on any surface, including microplastics (Wright et al., 2020). This work aimed to isolate and identify plastic-degrading microorganisms from plastic residues of the SAV biosphere reserve and quantify CEH activity expressed during the growth of microorganisms in the presence of polymer.

2 Materials and Methods

2.1 Sampling

Sampling was done on three SAV islands, "Sacrificios" (IS), "Salmedina" (ISM), and "Isla Verde" (IV). Decomposing plastics and other materials with microbial growth were taken in sterile conditions and transported to the analysis laboratory. Sampling was done following the Mexican norm PROY-NMX-AA-138-SCFI-2006.

2.2 Microorganisms isolation and selection

A representative portion of plastic samples were inoculated in liquid saline minimal medium (SMM) for bacteria reported by Paniagua-Meza (2008) and in minimal media (MM) for fungi, according to Hill and Käfer (2001), both containing NaCl (34 g L^{-1}). In the case of MM, the following slight modifications were done: glycerol (0.2%), glutamine (0.0073 g mL^{-1}), and methionine (0.0014 g mL^{-1}) sterilized by filtration were added after MM sterilization by autoclave. Bacteria were incubated for seven days for microbial adaptation at 37°C and fungi for 15 days at 28°C. Microorganisms from the adaptation step were inoculated in SMM or MM plates with PET from commercial water bottle residues sizing <1 mm (4 g L^{-1}) as the only carbon source for microbial selection. Microorganisms were incubated for 30 days for microbial adaptation at the temperatures mentioned above. The selected microorganisms were inoculated in nutritive agar plates for bacteria and potato dextrose agar (PDA) plates for fungi. Itraconazole (0.5 μg L^{-1}) was added for bacteria isolation and chloramphenicol (0.085 g mL^{-1}) for fungi isolation.

2.3 Microbial identification

Microbial culture plates were observed in a stereoscope (10X) for macroscopic identification. Microscopic identification was realized with Gram stain for bacteria (100X) and Bromophenol blue stain for fungi (40X).

Molecular identification was done by 16S rRNA and ITS amplicon sequencing. For this purpose, the microorganisms were inoculated in a nutritive (bacteria) or PDA (fungi) broth with agitation for 15 days at conditions mentioned in section 2.2. Bacteria were collected by centrifugation and mycelia by filtration; both cells were disrupted using a mortar and pestle in liquid nitrogen. DNA extraction was done, according to Ausubel et al. (1992). The 16S rRNA and ITS genes were amplified for polymerase chain reaction (PCR). The 16S primers for bacteria (P1: AGAGTTTGATCCTGGCTCAG; P3: AAGGAGGTGATCCAGCC) and ITS primers for fungi (ITS1: TCCGTAGGTGAACCTGCGG; ITS4: TCCTCCGCTTATTGATATGC) were used (Weisburg et al., 1991; White et al., 1990). DNA and PCR product concentration and purity were determined in the Epoch 2 microplate spectrophotometer (BioTek), and integrity was confirmed in 1% agarose gel electrophoresis. PCR amplicons were sequenced at the Biotechnology Institute (UNAM) using an automatic 16-capillary DNA sequencer 3130xl (Applied Biosystems). Obtained DNA sequences were analyzed in the Chromas program, aligned in the MultAlin program and compared with reported sequences in databases of the National Center for Biotechnology Information (NCBI) (Goodstadt and Ponting, 2001; Corpet, 1988).

2.4 Determination of PET degradation

2.4.1 Growth of microorganisms in the presence of PET

The PET degradation was evaluated by growing the microorganisms in 4 g L^{-1} of PET in 50 mL of MM or SMM liquid cultures using PET triturate from commercial water bottles (< 355 μm) generating PET microplastics (PET-MP) as a carbon source. The fungi cultures were inoculated with 1 $\times$ 10^6 spores mL^{-1}, and bacteria cultures were inoculated with 200 μL of liquid MM culture samples (DO 600 nm = 0.9). Bacteria were incubated for 8 days at 37°C and fungi for 15 days at 28°C in a stationary incubator. Samples were collected every 24 h for eight days in bacterial cultures and every 24 h for 15 days in fungi cultures for the following analysis. A control without microorganisms was evaluated under the same conditions.

2.4.2 Determination of polymer degradation

PET degradation was evaluated by dry weight measurement, titration, scanning electron microscopy (SEM) and infrared spectroscopy (IR), and data were normalized (Vázquez-Alcántara et al., 2021). For dry weight measurements, after hydrolysis, the residual PET-MP were washed with ethanol and dried in an oven at 50°C until constant weight before weight registration. For titration measurements, 1 mL of the medium was titrated with 0.01 N NaOH and phenolph-

thalein as an indicator, then the expenditure of NaOH was recorded. In both cases, a negative control was evaluated, corresponding to the hydrolysis reaction without the addition of microorganisms.

Scanning electron microscopy (SEM) of PET-MP observations was done in an FEI Sirion XL30 SEM instrument with a field emission gun (FEG-SEM). A thin layer of Au was deposited on the polymeric particles to avoid charge accumulation during the SEM observations. FTIR was performed on a Bruker Alpha spectrometer. The spectral output was recorded in transmittance mode, and 16 scans were acquired in the 400-4 400 cm^{-1} range and a resolution of 4 cm^{-1}. The resulting spectra were compared with reference polymers (standards) or those available in the Open Access Spectral Database (https://spectrabase.com/).

2.5 Enzymatic assays

2.5.1 Esterase activity measurement

The *p*-nitrophenyl acetate (*p*-NPA) was used as a substrate, and the esterase activity quantification was evaluated as described by Vázquez-Alcántara and coworkers (2021). Esterase activity in crude extracts from SMM and MM liquid cultures with PET-MP from commercial water bottles was determined following absorbance at 420 nm for 30 min every 5 min after *p*-NPA addition. An enzymatic activity unit (U) is the enzyme amount that transforms one μmol of *p*-NPA per minute.

2.5.2 Protein determination

Protein concentration was determined using the Bradford method (1976). Proteins were quantified according to the instructions provided by the manufacturer reagent (Bio-Rad). A standard curve of 0.01 mg to 0.1 mg of bovine serum albumin (BSA) was used.

3 Results

3.1 Samples collection

A brief description of the samples collected in SAV and the location of the collection sites is shown in Table 1. The samples had biofilms on the surface or were in contact with biofilms. Principally, pieces of various plastics and hydrocarbon residues were found.

3.2 Microorganisms isolation and selection

Microbial growth was observed in M2 ISM, M3 ISM, M1 IS, M1 IV, M4 IV, M5 IV and M6 IV samples after incubation in SMM and MM with a high concentration of salt (NaCl 34 g L^{-1}). Besides, after the microorganism's adaptation, microorganism growth was obtained in SMM or MM plates with PET as the only carbon source.

3.3 Microbial identification

3.3.1 Bacterial identification

The macroscopic and microscopic characteristics and the molecular identification of isolated bacteria are described in Table 2.

All macro- and microscopic descriptions are in accordance with the molecular identification. Bacteria were isolated from samples from the three assessed islands: one from Sacrificios, three from Salmedina, and three from Isla Verde. It is essential to mention that we have obtained two strains of *Aneurinibacillus migulanus* isolated from two different samples (M1 IS (red bottle cap) and M2 ISM (hydrocarbon fragment)) from different islands ("Sacrificios" and "Salmedina"). On the other hand, two strains of *Bacillus cereus* were obtained from two widely different samples (M1 IV (hydrocarbon residue) and M6 IV (white rigid plastic foam) from the same island ("Isla Verde"). *Bordetella petri* and *A. migulans* were isolated from the same hydrocarbon fragment collected on the "Salmedina" island. *Bacillus thuringesis* was isolated from different residues on the "Salmedina" island. *Lysinibacillus fusiformis* was collected from a white rigid thick plastic at "Isla Verde," and *Bacillus pacificus* was obtained from different residues (Tree bark, blue plastic bottle cap, white plastic lollipop stick) in "Isla Verde."

3.3.2 Fungal identification

One fungus was isolated from each island sampled from M2 IV, M2 ISM, and M3 IS samples. The macroscopic and microscopic characteristics of fungi and their molecular identification are described in Table 3, and all descriptions are in accordance. Interestingly, all identified fungi belonged to the Aspergillus genus.

In Figure 1, the microscopic view of *A. niger* isolated from the M3 IS sample can be observed; it shows a characteristic conidial head, where a conidiophore terminated by a vesicle with phialides, and conidia are observed.

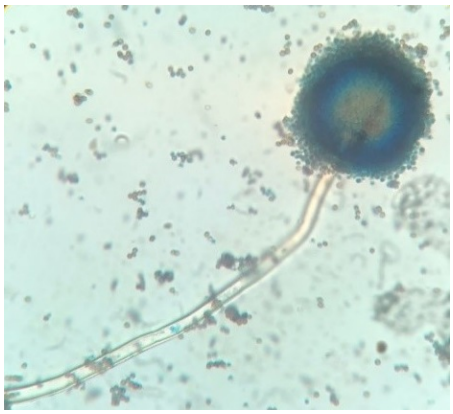


Figure 1. Microscopic view of *Aspergillus niger* (M3 IS sample) stained with bromophenol blue (40 X).

Table 1. Samples recollected in SAV

Sample ID	Ubication		Description	Image
"Sacrificios" island (IS)				
M1 IS	Latitude	19°10'27.09" N	Red and purple plastic bottle cap, solid hydrocarbon residue, piece of plastic straw	
	Longitude	96°05'34.43" O		
M2 IS	Latitude	19°10'30.36" N	Wood fragments with adherences similar to fungi	
	Longitude	96°05'33.83" O		
M3 IS	Latitude	19°10'28.92" N	Piece of plastic fork, hydrocarbon residues	
	Longitude	96°05'33.83" O		
M4 IS	Latitude	19°10'01.24" N	Piece of blue rigid plastic, red plastic bottle cap with hydrocarbon, white rigid plastic	
	Longitude	96°05'33.83" O		
"Salmedina" island (ISM)				
M1 ISM	Latitude	19°04'47.06" N	Piece of green rigid plastic	
	Longitude	95°57'19.05" O		
M2 ISM	Latitude	19°04'46.99" N	Hydrocarbon rigid fragment	
	Longitude	95°57'18.88" O		
M3 ISM	Latitude	19°12'00.05" N	Piece of marron expanded polystyrene, plastic bottle cap, piece of white rigid plastic, hydrocarbon fragment.	
	Longitude	96°04'02.49" O		
M4 ISM	Latitude	19°11'59.72" N	Piece of tubular plastic	
	Longitude	96°04'02.85" O		
M5 ISM	Latitude	19°12'02.46" N	Coral fragment, yellow thin rigid plastic, hydrocarbon residue	
	Longitude	95°04'02.30" O		
"Isla verde" island (IV)				
M1 IV	Latitude	19°12'02.96" N	Hydrocarbon rigid residue	
	Longitude	96°04'00.70" O		
M2 IV	Latitude	19°12'02.96" N	Piece of expanded polystyrene, white rigid and thin plastic	
	Longitude	96°04'00.70" O		
M3 IV	Latitude	19°12'01.86" N	Red plastic bottle cap	
	Longitude	96°03'59.84" O		
M4 IV	Latitude	19°12'01.86" N	White, rigid, thick plastic	
	Longitude	96°03'59.84" O		
M5 IV	Latitude	19°12'01.61" N	Tree bark, blue plastic bottle cap, white plastic lollipop stick	
	Longitude	96°03'59.64" O		
M6 IV	Latitude	19°12'01.61" N	Piece of white foam rigid plastic with green and brown adherences and hydrocarbon adherences and hydrocarbon	
	Longitude	96°03'59.64" O		

Table 2. Bacterial identification

Macroscopic characteristics						
Sample ID	**Bacterial colony aspect**					
	Form	**Border**	**Color**	**Surface**	**Consistency/texture**	**Elevation**
M1 IS	Circular	Undulate	Light brown	Wrinkled	Mucoid	Raised
M2 ISM 1	Irregular	Undulate	Dark brown	Smooth	Butyrose	Convex
M2 ISM 2	Circular	Enteri	Light brown	Wrinkled	Mucoid	Raised
M3 ISM	Circular	Enteri	Dark brown	Wrinkled	Mucoid	Convex
M1 IV	Circular	Undulate	Light brown	Smooth	Dry	Flat
M4 IV	Irregular	Undulate	Light brown	Smooth	Mucoid	Convex
M5 IV	Circular/ Fusifom	Lobete	Gray-Brown	Smooth	Butyrose	Convex
M6 IV	Circular	Enteri	Light brown	Smooth	Butyrose	Convex

Microscopic characteristics and molecular identification					
Sample ID	**Microscopic description**		**Molecular identification**		
	Morphology	**Gram**	**Microorganism**	**% Identity**	**E value**
M1 IS	Bacillus	+	*Aneurinibacillus migulanus*	99.54	0
M2 ISM 1	Coccus	–	*Bordetella petrii*	100	0
M2 ISM 2	Bacillus	+	*Aneurinibacillus migulanus*	99.79	0
M3 ISM	Bacillus	+	*Bacillus thuringiensis*	99.75	0
M1 IV	Bacillus	+	*Bacillus cereus*	99.58	0
M4 IV	Bacillus	+	*Lysinibacillus fusiformis*	99.52	0
M5 IV	Bacillus	+	*Bacillus pacificus*	99.86	0
M6 IV	Bacillus	+	*Bacillus cereus*	99.85	0

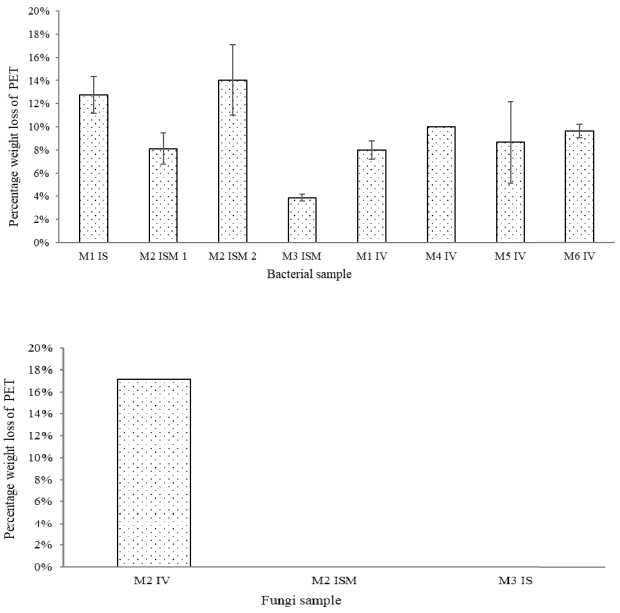


Figure 2. PET degradation determined by the percentage of weight loss after 8 or 15 days of growth in the polymer presence of bacterial and fungi strains, respectively. The results are the means from three replicates.

3.4 PET Biodegradation

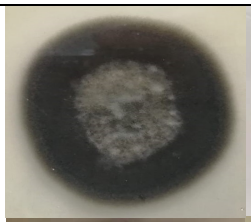
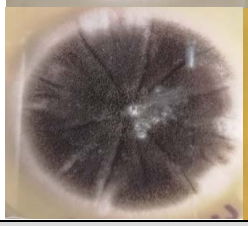
The ability of the isolated microorganisms to degrade PET-MP was evaluated. Figure 2 shows the PET weight loss percentage after the hydrolysis by the strains. Weight loss was observed in all bacteria treatments after eight days of culture; however, the highest values were obtained after the growth of the two isolated strains of *Aneurinibacillus migulanus* from samples (M1 IS and M2 ISM), over 10% of PET weight loss.

In fungus cultures, only *Aspergillus flavus* showed a weight loss of PET, about 17%. This result was confirmed by the acid titration of the crude extract (CE). The CE of *A. flavus* presented acidity after seven days of culture, which increased until fourteen days of incubation, having the highest value after 12 days of *A. flavus* growth (Figure 3). In the case of *A. oryzae* and *A. niger*, it is important to mention that a low PET degradation determined by acid titration was observed after 1 and 3 days, respectively (Figure 3).

Fig. 4 shows the SEM image of PET triturates after *Aneurinibacillus migulanus* hydrolysis; in contrast with the control (4A), porous regions can be observed after *A. migulanus* growth (4B). SEM images of PET treated with *A. flavus* showed holes and an eroded surface after fourteen days (Figure 4C & 4D).

Figure 5 shows the FTIR spectra for PET triturates without treatment (black) and PET treated with *Aspergillus flavus*

Table 3. Fungal identification

Macroscopic characteristics			
Sample ID	**Colony morphology**	**Image of petri dish with fungal colony**	
		Front view	**Reverse view**
M2 IV	Velvety, green, dark color		
M2 ISM	Velvety, white color		
M3 IS	Velvety, black and white color		

Microscopic characteristics and molecular identification				
Sample ID	**Microscopic description**	**Molecular identification**		
	Morphology	**Identification**	**% Identity**	**E value**
M2 IV	Mycelium is macrosiphonate, septate and hyaline and has long conidiophores, a circular vesicle with two series of phialides.	*Aspergillus flavus*	99.52	0
M2 ISM	Conidiophores are long, rough-walled, conidial head large, radiate with globose conidia.	*Aspergillus oryzae*	100.00	0
M3 IS	Mycelium is macrosiphonate, septate and hyaline, has long conidiophores, and a circular vesicle with two series of phialides at 360 ° angle, the first phialide series is extensive, and the second is small (Figure 1).	*Aspergillus niger*	99.08	0

(red). The spectra have differences in transmittance, especially in the carboxyl and hydroxyl functional groups (2 and 3), demonstrating the ester bond hydrolysis. The alkane chain (1) had just a 0.01 difference in transmittance, and the aromatic ring (4) did not show a difference.

3.5 CEH activity in crude extracts of isolated microorganisms growing in PET-MP presence

The leading group of enzymes reported as PET hydrolases is the carboxylic ester hydrolases (CEH), such as cutinases, lipases, and PETases (Vázquez-Alcántara et al., 2021; Yoshida et al., 2021; da Costa et al., 2020). In this study, we focus on determining the enzymatic activity of CEH when the microorganisms are in the presence of PET. Table 4 shows the maximum CEH activity detected in each crude extract of bacteria or fungi growing in PET presence as a sole carbon source during 8 and 15 days, respectively.

Table 4. Maximum CEH specific activity in crude extracts of bacteria and fungi growing in PET

Sample bacterial ID	Day of Culture	U mg^{-1} protein
Aneurinibacillus migulanus	1	36
Aneurinibacillus migulanus	1	82
Bacillus cereus	1	27
Lysinibacillus fusiformis	1	113
Bacillus pacificus	1	113
Bacillus cereus	1	160
Bacillus thuringiensis	4	90
	7	159
Bordetella petrii	7	255
Sample fungi ID	**Day of Culture**	**U mg^{-1} protein**
A. oryzae	8	780
A. niger	9	647
A. flavus	11	121
	13	266

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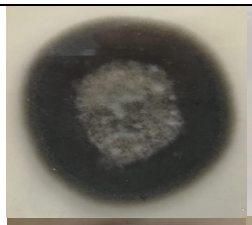
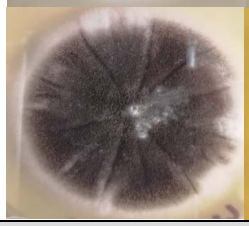
M2 IV	Velvety, green, dark color		
M2 ISM	Velvety, white color		
M3 IS	Velvety, black and white color		

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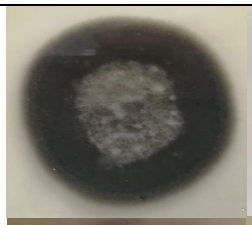
M2 IV	Velvety, green, dark color		
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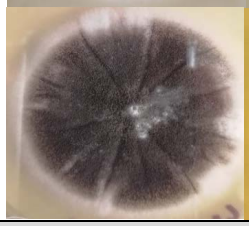
M3 IS	Velvety, black and white color		

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M2 IV	Mycelium is macrosiphonate, septate and hyaline and has long conidiophores, a circular vesicle with two series of phialides.	*Aspergillus flavus*	99.52	0
M2 ISM	Conidiophores are long, rough-walled, conidial head large, radiate with globose conidia.	*Aspergillus oryzae*	100.00	0
M3 IS	Mycelium is macrosiphonate, septate and hyaline, has long conidiophores, and a circular vesicle with two series of phialides at 360 ° angle, the first phialide series is extensive, and the second is small (Figure 1).	*Aspergillus niger*	99.08	0

(red). The spectra have differences in transmittance, especially in the carboxyl and hydroxyl functional groups (2 and 3), demonstrating the ester bond hydrolysis. The alkane chain (1) had just a 0.01 difference in transmittance, and the aromatic ring (4) did not show a difference.

3.5 CEH activity in crude extracts of isolated microorganisms growing in PET-MP presence

The leading group of enzymes reported as PET hydrolases is the carboxylic ester hydrolases (CEH), such as cutinases, lipases, and PETases (Vázquez-Alcántara et al., 2021; Yoshida et al., 2021; da Costa et al., 2020). In this study, we focus on determining the enzymatic activity of CEH when the microorganisms are in the presence of PET. Table 4 shows the maximum CEH activity detected in each crude extract of bacteria or fungi growing in PET presence as a sole carbon source during 8 and 15 days, respectively.

Table 4. Maximum CEH specific activity in crude extracts of bacteria and fungi growing in PET

Sample bacterial ID	Day of Culture	U mg^{-1} protein
Aneurinibacillus migulanus	1	36
Aneurinibacillus migulanus	1	82
Bacillus cereus	1	27
Lysinibacillus fusiformis	1	113
Bacillus pacificus	1	113
Bacillus cereus	1	160
Bacillus thuringiensis	4	90
	7	159
Bordetella petrii	7	255
Sample fungi ID	**Day of Culture**	**U mg^{-1} protein**
A. oryzae	8	780
A. niger	9	647
A. flavus	11	121
	13	266

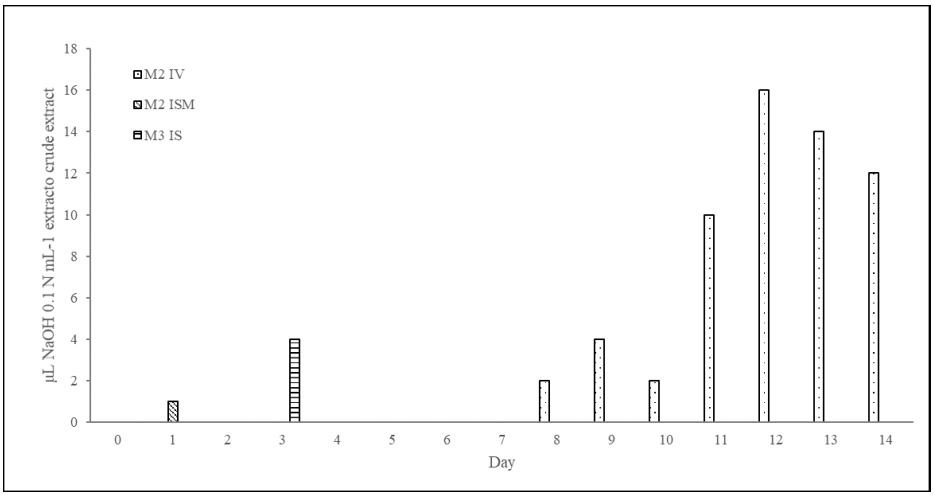


Figure 3. Kinetics of PET degradation by isolated fungi measured by acid titration of crude extracts.

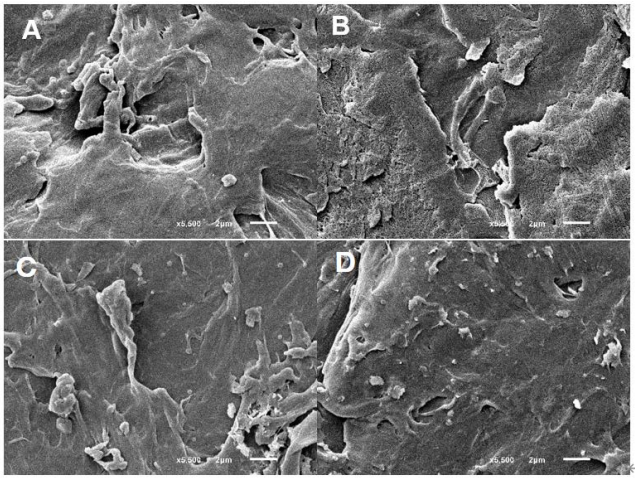


Figure 4. PET micrograph. (A) PET control (B) PET treated with *Aneurinibacillus migulanus* (C) PET control (D) PET treated with *Aspergillus flavus*, 5500X.

In the case of bacteria, most of them had significant CEH activity after 24 h, except for *B. thuringensis* and *B. petrii*. *B. thuringensis* showed two activity peaks at 4 and 7 days; despite this, the sample had a minor % of weight loss of PET (4%). *B. petrii* showed the maximum CEH activity on day 7, displaying 8% PET weight loss after eight days; additionally, this bacterium had the highest CEH activity from all bacteria isolated.

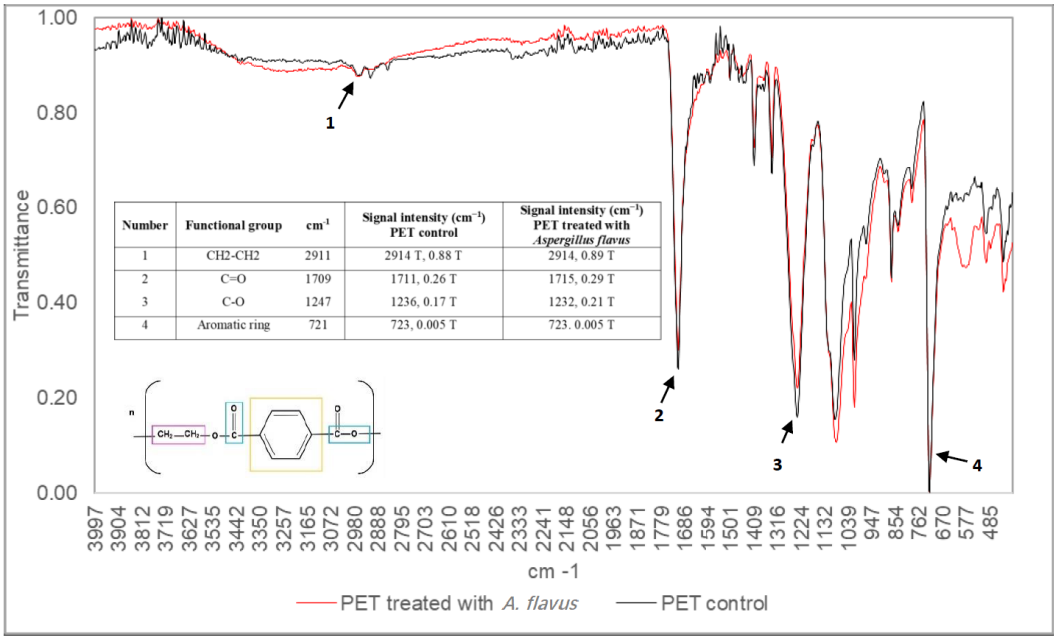
For fungi, the significant CEH activity was between 11 and 13 days (121 and 266 U mg^{-1}, respectively) in the crude extract of *A. flavus*; this agrees with the already mentioned PET biodegradation by *A. flavus*, where the highest value was obtained after 12 days. *A. oryzae* had the maximum enzymatic activity at eight days of culture (780 U mg^{-1}) followed by *A. niger* at nine (647 U mg^{-1}); however, despite the CEH activity being more significant than that of *A. flavus*, these fungi did not display observable PET biodegradation evidence determined by weight loss. Still, low PET degradation was observed by acid titration after 1 and 3 days, respectively (Figure 3).

4 Discussion and Conclusions

The samples where bacterial growth was identified in this study have a plastic or hydrocarbon waste origin. There are few reports on the degradation of plastic waste by bacteria of the genera found in this study, even more so when they are of marine origin. In 2012, Chaisu and coworkers reported *A. migulanus* isolated from soil in Taiwan as an organism with a degrading capacity of PLA, demonstrating in 2016 that the bacterium can degrade commercial bioplastic packaging (Chaisu, 2016).

On the other hand, *Bordetella petrii* isolated from a pesticide-degrading mixed bacterial culture in India has been reported as capable of degrading endosulfan and endosulfate by biosurfactant-producing bacteria and 1,2,4-trichlorobenzene (Odukkathil et al., 2015; Wang et al., 2007). *B. petrii* has been isolated from marine sponges, river sediment, and polluted soil; it encodes genes involved in aromatic compound degradation and detoxification of heavy metals (Gross et al., 2008; Lechner et al., 2009). It is important to mention the research of Kim and Park (2010), where *B. petrii* isolated from the soil in Korea is reported as a degrader of PLA, a bioplastic. Besides, El Awady et al. (2022) reported that the *B. petrii* bacterium is a degrader of the polymer polyurethane (PU).

Ray (2019) reported *Bacillus thuringiensis* as a bacterium capable of low-density polyethylene (LDPE) biodegradation individually and in conjunction with *Lysinibacillus sphaericus*; both bacteria were obtained from Carolina Biological Supply Company. Interestingly, Roberts et al. (2020) informed that a consortium of five strains containing *Bacillus thuringiensis* grew with PET as a carbon source; they were collected six inches beneath the topsoil layer from the Gulf Coast of southeast Texas within the greater Houston area



Number	Functional group	cm-1	Signal intensity (cm⁻¹) PET control	Signal intensity (cm⁻¹) PET treated with *Aspergillus flavus*
1	CH2-CH2	2911	2914 T, 0.88 T	2914, 0.89 T
2	C=O	1709	1711, 0.26 T	1715, 0.29 T
3	C-O	1247	1236, 0.17 T	1232, 0.21 T
4	Aromatic ring	721	723, 0.005 T	723. 0.005 T

Figure 5. FTIR spectra of PET treated with *A. flavus* (red) and PET control (black).

and near the shoreline at East Beach in Galveston. Other kinds of contaminants can be degraded effectively by *Bacillus thuringiensis* as cypermethrin, used as an insecticide (Bhatt et al., 2020), and chlorpyrifos, used as an organophosphate pesticide (Ambreen et al., 2021).

Bacillus cereus isolated from sediment samples of mangrove sites in the east, west, south, and north of Peninsular Malaysia has scientific evidence about its capacity to grow with different microplastic polymers as the sole carbon source, such as PET, PS, and polyethylene (PE) (Auta et al., 2017; Suresh et al., 2011). Additionally, Arefian et al. (2020) identified lipase and amylase activity during polycarbonate degradation with *B. cereus*.

Lysinibacillus fusiformis is a reported bacterium degrader of aflatoxin B1 (Adebo et al., 2016); petroleum hydrocarbons (Li et al., 2020) and LDPE generally used for plastic bags, where, *L. fusiformis* was collected from the wastewater collected from the Bangur area of Kolkata Municipal Corporation (Mukherjee et al., 2016; Kalia et al., 2022) and PP (Jeon et al., 2021).

B. pacificus has been reported as an effective halotolerant bacterial biodegrading naphthalene; it was isolated from an oil-spilled contaminated site from Ennore Beach, Chennai, Tamil Nadu, India (Bhandari et al., 2021). Additionally, a cellulase from this strain was studied by Krishnaswamy and coworkers in 2022; it was demonstrated that the enzyme could degrade LDPE.

In resume, all the bacteria genera found in this study have been reported for the plastic degradation capacity of other polymers than PET: *Bordetella petrii* (PLA), *Aneurinibacillus migulanus* (PLA), *Bacillus thuringiensis* (LDPE and other pollutants), *Bacillus cereus* (PS and PE), *Lysinibacillus fusiformis* (LDPE) and *Bacillus pacificus* (LDPE). Additionally, some of these bacteria genera have degradation evidence in the literature of other pollutants; *Lysinibacillus fusiformis* is a degrader of aflatoxins, and *Bordetella petrii* is reported as an endosulfan and heavy metals degrader.

On the other hand, fungi have been reported for the biodegradation of plastics (Zeghal et al., 2021). It is known that they produce enzymes such as cutinases that can hydrolyze polyesters found in plastic residues (Vázquez-Alcántara et al., 2021; Ahmaditabatabaei et al., 2021). They usually have been isolated from terrestrial environments, and few marine fungi for plastic degradation have been reported; indeed, as many fungi are ubiquitous, having a clear definition of "marine fungus" is challenging (Zeghal et al., 2021). Lacerda et al. (2020), found that the genus Aspergillus was the most abundant after studying the fungal diversity associated with plastics in the surface waters of the Western South Atlantic and the Antarctic Peninsula. In this sense, it is important to mention that all the marine fungi isolated in this study belonged to the Aspergillus genera.

In general, fungal isolates exhibited higher CEH activity compared to bacteria, being the fungi *A. oryzae* and *A. niger* with the highest activity. We found an observable PET degradation by *A. flavus* after 11 days using the weight loss technique, which agrees with the maximum CEH activity found after 11 days of fungi growth on PET presence. In the case of *A. oryzae* and *A. niger*, a high CEH activity was found only during the first days of growth (1 and 3 days, respectively), which could be related to the low PET degradation observed by acid titration after 8 and 9 days, afterward, probably the CEH activity is inhibited by the reaction products. The PET degradation by these two Aspergillus species must be deeply studied to determine better polymer degradation conditions and identify the induced CEH to characterize their

inhibition and stability during PET degradation.

Aspergillus flavus can utilize PU as the sole carbon source (Khan et al., 2017); Mathur and Prasad (2012) identified an extracellular esterase activity possibly responsible for PU degradation. *A. flavus* was obtained from soil samples collected from plastic waste disposal sites from Uttaranchal, India. Zhang et al. (2020) reported the degradation of high-density polyethylene (HDPE) microplastic by *A. flavus* isolated from the guts of the wax moth *Galleria mellonella*, probably by laccase activity. Maheswaran et al. (2023) reported a strain of *A. flavus* and *A. niger* isolated from plastic waste showing PET film degradation.

Ogunbayo et al. (2019) isolated *Aspergillus niger* from the soil of a dump site at the University of Lagos, Nigeria, and Raaman et al. (2012) isolated it from PE bags in polluted areas of Chennai, Tamil Nadu, demonstrating the degradation of LDPE by *A. niger*. This fungus can also degrade PLA when it colonizes the surface and causes cracks and depression (Chai et al., 2022).

Aspergillus oryzae can hydrolyze the biodegradable plastic bags of poly (butylene succinate-co-adipate) (PBSA) by a cutinase (Tanaka and Gomi, 2021). Jayaprakash and Palempalli (2018) studied the process of HDPE and LDPE degradation with *A. oryzae* isolated from the polyethylene bags buried in the soil for six months in India.

Our results are similar to those recently reported by Maheswaran and coworkers (2023), who isolated *A. flavus* from sediment samples contaminated with microplastics from a stretch of the Kaveri River in South India. However, they demonstrated that *A. flavus* displayed a PET film degraded by a 16% weight loss after 50 days.

Microorganisms do not have very high percentages of degradation. Still, we can claim that they express the enzymes necessary for biodegradation, indicating that it is vital to investigate the degradation pathways and enzymes involved. Interestingly, CEH activity was found after microorganisms' growth in PET presence and to this class belongs many identified PET-degrading enzymes such as cutinases, PET-ases, lipases and esterases. Consequently, identifying these enzymes and transcriptomic and proteomic studies during PET-degradation by isolated bacteria and fungi are of great interest.

Research on marine microorganisms for waste plastic biodegradation is scarce despite the high plastic pollution of oceans. Twelve marine microorganisms, eight bacteria, and three fungi were isolated from biofilms of plastic residues from the SAV biosphere reserve. All the bacteria and one fungus showed evidence of degraded PET-MP from water bottles. Besides, CEH activity was detected in crude extracts of these microorganisms growing in the presence of PET. There are few studies of PET degradation with bacteria or fungi of marine origin, and even fewer isolated from decomposing plastic waste. Since these microorganisms were isolated from the plastic waste of different polymers other than PET, it is possible that they can degrade more than one plastic waste polymer.

5 Perspectives

A deeper study of PET residue degradation using the isolated marine strains and evaluating the degradation of other polymers such as polyethylene, polypropylene, and polystyrene must be done. Besides, the polyester degraded in different salt concentrations must be estimated. Investigating transcriptomics and proteomics, primarily of fungus, is another crucial step in determining the metabolic pathways and the polymer breakdown enzymes.

Acknowledgments

CONAHCyT for financial support with project A1-S-47929. LNO and LVA are recipients of a Ph.D. CONAHCyT fellowship (858149 and 731784). FFR and MLAJ are recipients of an M.Sc. CONAHCyT fellowship (991025 and 1142755). We thank Antonia G. Rodríguez-Lagunes for the Graphical Abstract design.

Declaration in the writing process

While preparing this work, the authors used Grammarly to improve manuscript writing. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

Conflict of Interest

The authors declare no competing interests.

References

Adebo, O.A., Njobeh, P.B., and Mavumengwana, V., 2016. Degradation and detoxification of AFB1 by Staphylococcoccus warneri, Sporosarcina sp. and *Lysinibacillus fusiformis*. Food Control, 68: 92-96. https://doi.org/10.1016/j.foodcont.2016.03.021

Ahmaditabatabaei, S., Kyazze, G., Iqbal, H.M., and Keshavarz, T., 2021. Fungal enzymes as catalytic tools for polyethylene terephthalate (PET) degradation. Journal of Fungi, 7(11): 931. https://doi.org/10.3390/jof7110931

Ambreen, S. and Yasmin, A., 2021. Novel degradation pathways for Chlorpyrifos and 3, 5, 6-Trichloro-2-pyridinol degradation by bacterial strain *Bacillus thuringiensis* MB497 isolated from agricultural fields of Mianwali, Pakistan. Pesticide Biochemistry and Physiology, 172: 104750. https://doi.org/10.1016/j.pestbp.2020.104750

Amobonye, A., Bhagwat, P., Singh, S., and Pillai, S., 2020. Plastic biodegradation: frontline microbes and their enzymes. Science of The Total Environment, 759: 143536. https://doi.org/10.1016/j.scitotenv.2020.143536

Arefian, M., Tahmourespour, A., and Zia, M., 2020. Polycarbonate biodegradation by newly isolated Bacillus strains. Archives of Environmental Protection, 46(1): 14-20. https://doi.org/10.24425/aep.2020.132521

Asiandu, A.P., Wahyudi, A., and Sari, S.W., 2020. A review: plastics waste biodegradation using plastics-degrading bacteria. Journal of Environmental Treatment Techniques, 9(1): 148-157. https://doi.org/10.47277/JETT/9(1)157

Auta, H.S., Emenike, C.U., and Fauziah, S.H., 2017. Screening of Bacillus strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation. Environmental Pollution, 231: 1552-1559. https://doi.org/10.1016/j.envpol.2017.09.043

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., and Seidman, J.G., 1992. Short protocols in molecular biology

Bhandari, R., Harsha Vardhan, K., Kumar, P.S., and Gayathri, K.V., 2021. Effective removal of naphthalene from contaminated soil using halotolerant bacterial strains and vermiremediation techniques. International Journal of Environmental Analytical Chemistry, 103(4): 761-778. https://doi.org/10.1080/03067319.2020.1863390

Bhatt, P., Huang, Y., Zhang, W., Sharma, A., and Chen, S., 2020. Enhanced cypermethrin degradation kinetics and metabolic pathway in *Bacillus thuringiensis* strain SG4. Microorganisms, 8(2): 223. https://doi.org/10.3390/microorganisms8020223

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Chai, X., He, C., Liu, Y., Niyitanga, E., Zhou, Y., Wang, L., and Zhang, W., 2022. Degradation of wheat straw/polylactic acid composites by *Aspergillus niger*. Polymer Composites, 43(3): 1823-1831. https://doi.org/10.1002/pc.26500

Chaisu, K., 2016. Study of plastic degrading bacteria by *Aneurinibacillus migulanus*. In 2016 Management and Innovation Technology International Conference (MITicon) (pp. MIT-209). IEEE. https://doi.org/10.1109/MITICON.2016.8025218

Chaisu, K., Charles, A.L., Guu, Y., and Chiu, C., 2012. Optimization of Poly Lactic Acid (PLA) plastic degradation by *Aneurinibacillus migulanus* using response surface methodology. International Proceedings of Chemical, Biological and Environmental Engineering (IPCBEE), 40, 22-27. ISSN: 2010-4618

Cházaro-Olvera, S., Morán-Silva, Á., Chávez-López, R., Vázquez-López, H., and Rodríguez-Varela, A.D.C., 2020. Density of mysis of Penaeus aztecus Ives, 1891, on a reef in the Southwest of the Gulf of Mexico. Hidrobiolgica, 30(3): 233-242. https://doi.org/:10.24275/uam/izt/dcbs/hidro/2020v30n3/

Corpet, F., 1988. Multiple sequence alignment with hierarchical clustering. Nucleic acids research, 16(22): 10881-10890. https://doi.org/10.1093/nar/16.22.10881

da Costa, A.M., de Oliveira Lopes, V.R., Vidal, L., Nicaud, J.M., de Castro, A.M., and Coelho, M.A.Z., 2020. Poly (ethylene terephthalate)(PET) degradation by Yarrowia lipolytica: Investigations on cell growth, enzyme production and monomers consumption. Process Biochemistry, 95: 81-90. https://doi.org/10.1016/j.procbio.2020.04.001

Danso, D., Chow, J., and Streit, W.R., 2019. Plastics: environmental and biotechnological perspectives on microbial degradation. Applied and environmental microbiology, 85(19): e01095-19. https://doi.org/10.1128/AEM.01095-19.

DOF (Diario Oficial de la Federacin). 1992. Decreto por el que se declara área natural protegida con el carácter de Parque Marino Nacional, la zona conocida como Sistema Arrecifal Veracruzano, ubicada frente a las Costas de los municipios de Veracruz, Boca del Río y Alvarado del estado de Veracruz Llave, con superficie de 52,238 hectáreas. Comisión Nacional de Áreas Naturales Protegidas (CONANP). https://simec.conanp.gob.mx/pdf_decretos/135_decreto.pdf

Dussud, C., Hudec, C., George, M., Fabre, P., Higgs, P., Bruzaud, S., and Ghiglione, J.F., 2018. Colonization of non-biodegradable and biodegradable plastics by marine microorganisms. Frontiers in microbiology, 9: 1571. https://doi.org/10.3389/fmicb.2018.01571

El Awady, M.E., Asker, M.S., Haggag, K.M., Abdelaziz, D., and El-Shall, F.N., 2022. Chemical and Biological Assay for The Degradation of Microwave Synthesized-Hyperbranched Poly (Urethane-Urea) By Local Bacterial Isolates. Egyptian Journal of Chemistry, 65(2): 297-30. https://doi.org/10.21608/ejchem.2021.81877.4042

Escamilla-Pérez, B.E., Ortiz-Lozano, L., Molina-Rosales, D.O., and Espinoza-Tenorio, A., 2021. Cultural importance of marine resources subject to fishing exploitation in coastal communities of Southwest Gulf of Mexico. Ocean and Coastal Management, 208: 105605. https://doi.org/10.1016/j.ocecoaman.2021.105605

Goodstadt, L., and Ponting, C.P., 2001. CHROMA: consensus-based colouring of multiple alignments for publication. Bioinformatics, 17(9): 845-846. https://doi.org/10.1093/bioinformatics/17.9.845

Gross, R., Guzman, C.A., Sebaihia, M., Martins dos Santos, V.A., Pieper, D.H., Koebnik, R., Martinez-Arias, R., 2008. The missing link: *Bordetella petrii* is endowed with both the metabolic versatility of environmental bacteria and virulence traits of pathogenic Bordetellae. BMC genomics, 9: 1-14. https://doi.org/10.1186/1471-2164-9-449

Jayaprakash, V. and Palempalli, U.M.D., 2018. Effect of palmitic acid in the acceleration of polyethylene biodegradation by *Aspergillus oryzae*. J Pure Appl Microbiol, 12: 2259-2268. https://doi.org/10.22207/JPAM.12.4.66

Jeon, J.M., Park, S.J., Choi, T.R., Park, J.H., Yang, Y.H., and Yoon, J.J., 2021. Biodegradation of polyethylene and polypropylene by Lysinibacillus species JJY0216 isolated from soil grove. Polymer Degradation and Stability, 191: 109662. https://doi.org/10.1016/j.polymdegradstab.2021.109662

Hill, T.W. and Kafer, E., 2001. Improved protocols for Aspergillus minimal medium: trace element and minimal medium salt stock solutions. Fungal Genetics Reports, 48(1): 20-21. https://doi.org/10.4148/1941- 4765.1173

Kalia, A. and Dhanya, M.S., 2022. Evaluation of biodegradation efficiency of xylene pretreated polyethylene wastes by isolated *Lysinibacillus fusiformis*. Nature Environment and Pollution Technology, 21(3): 1375-1380. https://doi.org/10.46488/NEPT.2022.v21i03.045

Khan, S., Nadir, S., Shah, Z.U., Shah, A.A., Karunarathna, S.C., Xu, J. and Hasan, F., 2017. Biodegradation of polyester polyurethane by Aspergillus tubingensis. Environmental pollution, 225: 469-480. https://doi.org/10.1101/2020.06.25.170654

Kim, M.N., and Park, S.T., 2010. Degradation of poly (Llactide) by a mesophilic bacterium. Journal of applied polymer science, 117(1): 67-74. https://doi.org/10.1002/app.31950

Krishnaswamy, V.G., Sridharan, R., Kumar, P.S., and Fathima, M.J., 2022. Cellulase enzyme catalyst producing bacterial strains from vermicompost and its application in low-density polyethylene degradation. Chemosphere, 288: 132552. https://doi.org/10.1016/j.chemosphere.2021.132552

Lacerda, A.L.D.F., Proietti, M.C., Secchi, E.R., and Taylor, J.D., 2020. Diverse groups of fungi are associated with plastics in the surface waters of the Western South Atlantic and the Antarctic Peninsula. Mole. Ecol. 2020: 15444. https://doi.org/10.1111/mec.15444

Lechner, M., Schmitt, K., Bauer, S., Hot, D., Hubans, C., Levillain, E. and Gross, R., 2009. Genomic island excisions in *Bordetella petrii*. BMC microbiology, 9: 1-11. https://doi.org/10.1186/1471-2180-9-141

Li, S.W., Huang, Y.X., and Liu, M.Y., 2020. Transcriptome profiling reveals the molecular processes for survival of *Lysinibacillus fusiformis* strain 15-4 in petroleum environments. Ecotoxicology and Environmental Safety, 192: 110250. https://doi.org/10.1016/j.ecoenv.2020.110250

Mapel-Hernández, M.D., Armstrong-Altrin, J.S., Botello, A.V., and Lango-Reynoso, F., 2021. Bioavailability of Cd and Pb in sediments of the National Park Veracruz Reef System, Gulf of Mexico. Applied Geochemistry, 133: 105085. https://doi.org/10.1016/j.apgeochem.2021.105085

Martinez, M.L., Silva, R., Lithgow, D., Mendoza, E., Flores, P., Martnez, R., and Cruz, C., 2017. Human impact on coastal resilience along the coast of Veracruz, Mexico. Journal of Coastal Research, (77 (10077)): 143-153. https://doi.org/10.2112/SI77-015.1

Mathur, G. and Prasad, R., 2012. Degradation of polyurethane by *Aspergillus flavus* (ITCC 6051) isolated from soil. Applied biochemistry and biotechnology, 167: 1595-1602. https://doi.org/10.1007/s12010-012-9572-4

Maheswaran, B., Al-Ansari, M., Al-Humaid, L., Raj, J.S., Kim, W., Karmegam, N., and Rafi, K.M., 2023. In vivo degradation of polyethylene terephthalate using microbial isolates from plastic polluted environment. Chemosphere, 310: 136757. https://doi.org/10.1016/j.chemosphere.2022.136757

Montoya-Mendoza, J., Alarcón-Reyes, E., Castañeda-Chávez, M.D.R., Lango-Reynoso, F., and Zamudio-Alemán, R.E., 2019. Heavy metals in muscle tissue of Pterois volitans from the Veracruz reef system national park, Mexico. International journal of environmental research and public health, 16(23): 4611. https://doi.org/10.3390/ijerph16234611

Mukherjee, S., Chowdhuri, U.R., and Kundu, P.P., 2016. Bio-degradation of polyethylene waste by simultaneous use of two bacteria: Bacillus licheniformis for production of bio-surfactant and *Lysinibacillus fusiformis* for bio-degradation. RSC advances, 6(4): 2982-2992. https://doi.org/10.1039/C5RA25128A

Narciso-Ortiz, L., Coreño-Alonso, A., Mendoza-Olivares, D., Lucho-Constantino, C.A., and Lizardi-Jiménez, M.A., 2020. Baseline for plastic and hydrocarbon pollution of rivers, reefs, and sediment on beaches in Veracruz State, Mxico, and a proposal for bioremediation. Environmental Science and Pollution Research, 27(18): 23035-23047. https://doi.org/10.1007/s11356-020-08831-z

Odukkathil, G. and Vasudevan, N., 2015. Biodegradation of endosulfan isomers and its metabolite endosulfate by two biosurfactant producing bacterial strains of *Bordetella petrii*. Journal of Environmental Science and Health, Part B, 50(2): 81-89. https://doi.org/10.1080/03601234.2015.975596

Ogunbayo, A.O., Olanipekun, O.O., and Adamu, I.A., 2019. Preliminary studies on the microbial degradation of plastic waste using *Aspergillus niger* and Pseudomonas sp. Journal of Environmental Protection, 10(5):625-631. https://doi.org/10.4236/jep.2019.105037

Paniagua-Meza, D., 2008. Aislamiento y caracterizacin de bacterias halfilas degradadoras de hidrocarburos. Master's thesis in biochemical sciences. UNAM.

Ramírez-Macías, J.I., Liaño-Carrera, F., Camarena-Luhrs, T., Gómez-Barrero, A.E. and Salas-Monreal, D., 2018. Environmental monitoring and management during the construction dredging for the first stage of New Port of Veracruz, Mexico, In Proceedings of the Western Dredging Association Dredging Summit and Expo 18. Western Dredging Association (WEDA). ISBN: 9781510867574

Raaman, N., Rajitha, N., Jayshree, A., and Jegadeesh, R., 2012. Biodegradation of plastic by *Aspergillus* spp. isolated from polythene polluted sites around Chennai. Journal of academia and industrial research, 1(6): 313-316.

Ray, R., 2019. The Effects of *Bacillus thuringiensis* and *Lysinibacillus sphaericus* on Low-Density Polyethylene. Available at SSRN 3586055.

PROY-NMX-AA-138-SCFI-2006. Proyecto de norma mexicana Residuos obtencin de muestras representativas, su manejo, conservacin y transporte-especificaciones, 2006.

Roberts, C., Edwards, S., Vague, M., León-Zayas, R., Scheffer, H., Chan, G., and Mellies, J.L., 2020. Environmental consortium containing pseudomonas and bacillus species synergistically degrades polyethylene terephthalate plastic. Msphere, 5(6): e01151-20. https://doi.org/10.1128/mSphere.01151-20.

Sigurgísladóttir, S., Konráòsdóttir, M., Jónsson, Á., Kristjnsson, J.K., and Matthiasson, E., 1993. Lipase activity of thermophilic bacteria from Icelandic hot springs. Biotechnology Letters, 15(4): 361-366. https://doi.org/10.1007/BF00128277

SISR (Servicio de Informacin sobre Sitios Ramsar), 2004. Parque Nacional Sistema Arrecifal Veracruzano. https://rsis.ramsar.org/es/ris/1346?language=es

Suresh, B., Maruthamuthu, S., Palanisamy, N., Ragunathan, R., Pandiyaraj, K.N., and Muralidharan, V.S., 2011. Investigation on biodegradability of polyethylene by *Bacillus cereus* strain Ma-Su isolated from compost soil. International Journal Of Medical Microbiology, 2: 292-302. http://www.interesjournals.org/IRJM

Tanaka, M., and Gomi, K., 2021. Induction and repression of hydrolase genes in *Aspergillus oryzae*. Frontiers in Microbiology, 12: 677603. https://doi.org/10.3389/fmicb.2021.677603

UNESCO (United Nations Educational, Scientific and Cultural Organization), 2006. Biosphere reserves in Latin America and the Caribbean. https://en.unesco.org/biosphere/lac

Vázquez-Alcntara, L., Oliart-Ros, R.M., García-Bórquez, A., and Peña-Montes, C., 2021. Expression of a Cutinase of *Moniliophthora roreri* with Polyester and PET-plastic residues degradation activity. Microbiology Spectrum, 9(3): e00976-21. https://doi.org/10.1128/Spectrum.00976-21.

Wang, F., Grundmann, S., Schmid, M., Dörfler, U., Roherer, S., Munch, J.C. and Schroll, R., 2007. Isolation and characterization of 1, 2, 4-trichlorobenzene mineralizing Bordetella sp. and its bioremediation potential in soil. Chemosphere, 67(5): 896-902. https://doi.org/10.1016/j.chemosphere.2006.11.019

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of bacteriology, 173(2): 697-703.

White, J.T., Bruns, T, Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. En PCR protocols: A guide to methods and applications, 2: 13-20. Academic Press. ISBN 0-12-372180-6

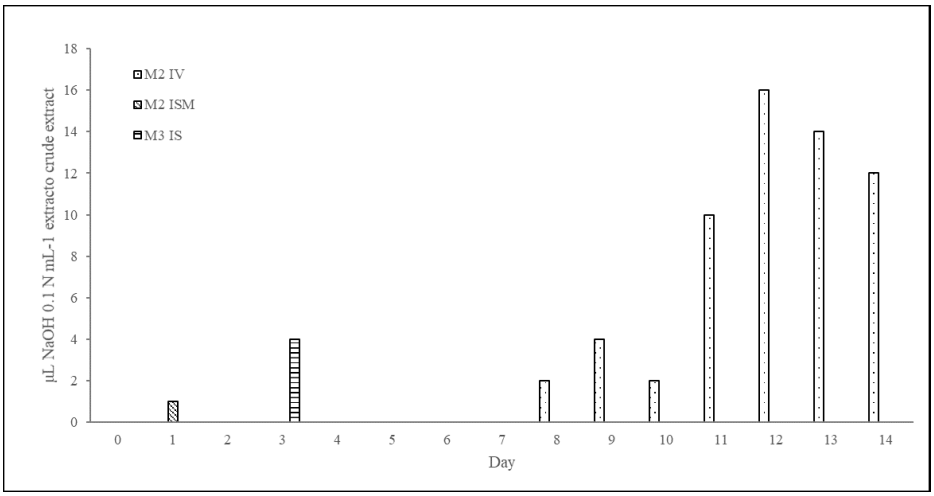
Wright, R.J., Erni-Cassola, G., Zadjelovic, V., Latva, M., and Christie-Oleza, J.A., 2020. Marine plastic debris: a new surface for microbial colonization. Environmental Science and Technology, 54(19): 11657-11672. https://doi.org/10.1021/acs.est.0c02305

Yoshida, S., Hiraga, K., Taniguchi, I., and Oda, K., 2021. *Ideonella sakaiensis*, PETase, and MHETase: From identification of microbial PET degradation to enzyme characterization. In Methods in enzymology, 648: 187-205. Academic Press. https://doi.org/10.1016/bs.mie.2020.12.007

Zeghal, E., Vaksmaa, A., Vielfaure, H., Boekhout, T., and Niemann, H., 2021. The potential role of marine fungi in plastic degradationa review. Frontiers in Marine Science, 8: 738877. https://doi.org/10.3389/fmars.2021.738877

Zhang, J., Gao, D., Li, Q., Zhao, Y., Li, L., Lin, H. and Zhao, Y., 2020. Biodegradation of polyethylene microplastic particles by the fungus *Aspergillus flavus* from the guts of wax moth *Galleria mellonella*. Science of the Total Environment, 704: 135931. https://doi.org/10.1016/j.scitotenv.2019.135931

Zhao, X., Boruah, B., Chin, K.F., oki, M., Modak, J.M., and Soo, H.S., 2022. Upcycling to sustainably reuse plastics. Advanced Materials, 34(25): 2100843. https://doi.org/10.1002/adma.202100843



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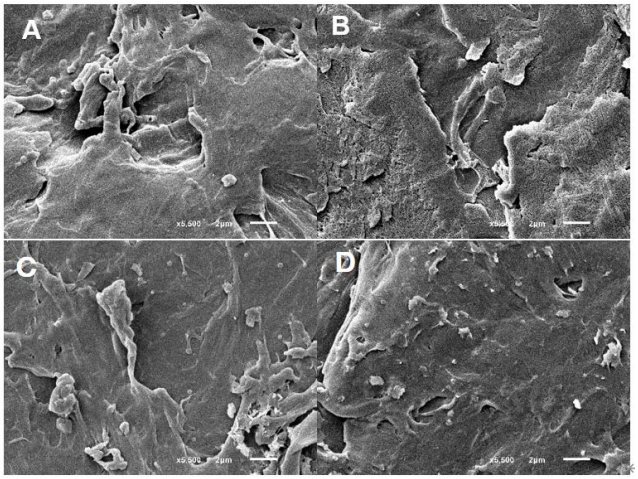

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4 Discussion and Conclusions

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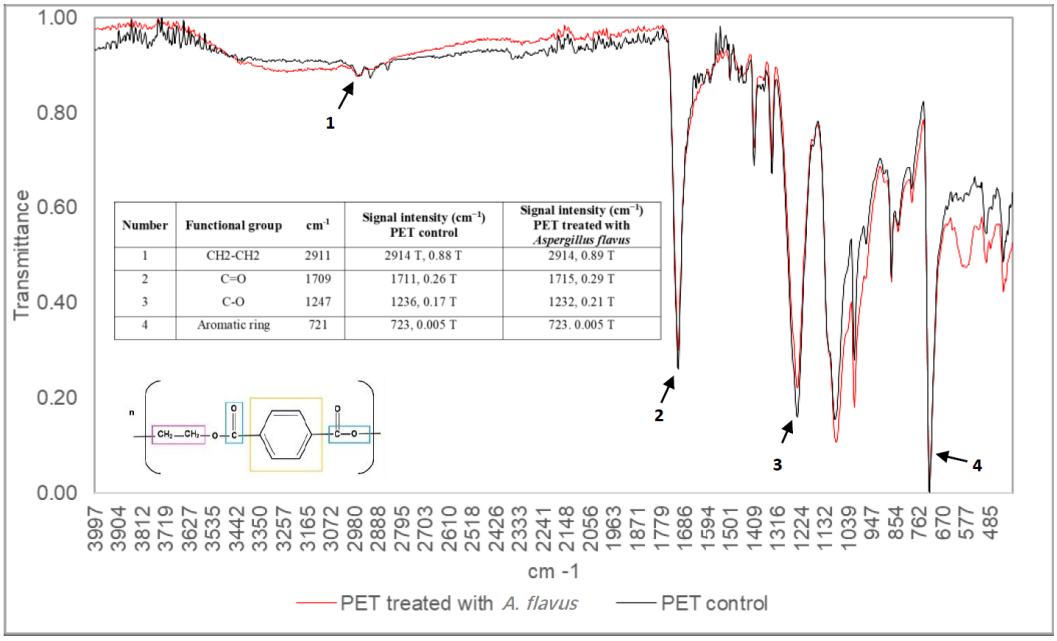
On the other hand, *Bordetella petrii* isolated from a pesticide-degrading mixed bacterial culture in India has been reported as capable of degrading endosulfan and endosulfate by biosurfactant-producing bacteria and 1,2,4-trichlorobenzene (Odukkathil et al., 2015; Wang et al., 2007). *B. petrii* has been isolated from marine sponges, river sediment, and polluted soil; it encodes genes involved in aromatic compound degradation and detoxification of heavy metals (Gross et al., 2008; Lechner et al., 2009). It is important to mention the research of Kim and Park (2010), where *B. petrii* isolated from the soil in Korea is reported as a degrader of PLA, a bioplastic. Besides, El Awady et al. (2022) reported that the *B. petrii* bacterium is a degrader of the polymer polyurethane (PU).

Ray (2019) reported *Bacillus thuringiensis* as a bacterium capable of low-density polyethylene (LDPE) biodegradation individually and in conjunction with *Lysinibacillus sphaericus*; both bacteria were obtained from Carolina Biological Supply Company. Interestingly, Roberts et al. (2020) informed that a consortium of five strains containing *Bacillus thuringiensis* grew with PET as a carbon source; they were collected six inches beneath the topsoil layer from the Gulf Coast of southeast Texas within the greater Houston area



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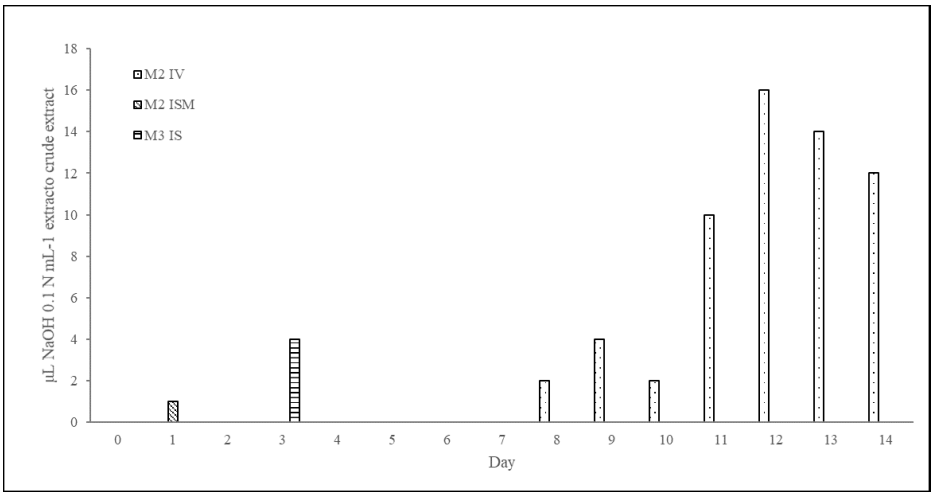

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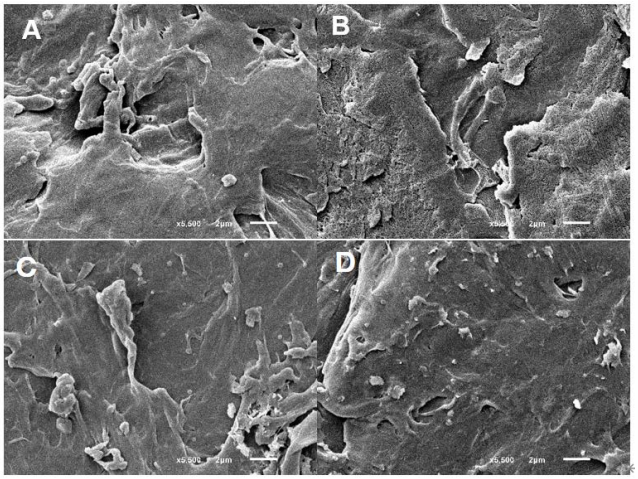

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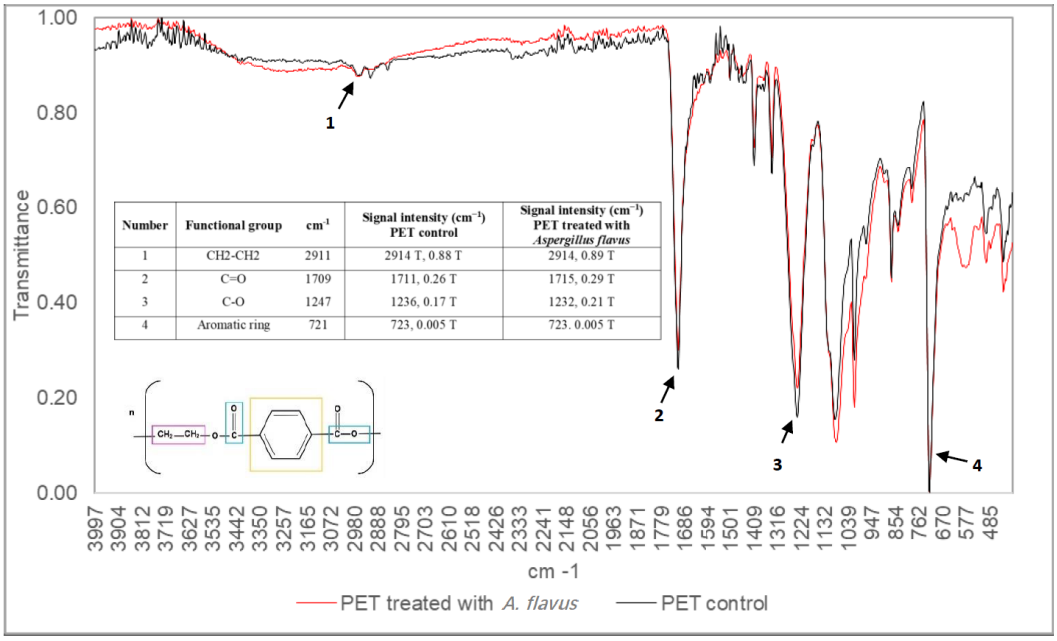

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