



Aiding the Environment: Microorganisms for the Degradation of Plastic in Soil and Water

M.S. Gabriel Salazar Robles^{1*} M.S. José María Cunill¹

¹Biotechnology Department, Metropolitan Polytechnic University of Puebla

Abstract: Biodegradation is a viable alternative to solve the serious problem of plastics in the environment, and it consists of the use of living organisms for the degradation of contaminants. Microorganisms are an ideal alternative to carry out biodegradation due to their ubiquity in many niches in addition to performing a complete plastic removal, even microplastics. There are many genera of bacteria and fungi with the metabolic armament to carry out this removal and its study has been extended for several decades, being a point of interest for various investigations due to the recent boom in sustainable development. The mechanism for the biodegradation of plastics is to break the polymer into smaller subunits in order to be assimilated by the microorganism, this is achieved through chemical alterations of the polymer. It is important to spread the knowledge involved in plastic biodegradation to increase the development of suitable techniques for the mitigation of this growing worldwide problem. A compilation of genera and species of bacteria and fungi that have the potential to degrade plastic is presented, showing its removal percentage in different growth conditions and for different kinds of non-biodegradable plastics. Also, the degradation metabolism is presented.

Keywords: biodegradation, plastic, microplastics, metabolism, bacteria, fungi

The problem of plastics

Treatment issues with plastics

Compared to organic products that take weeks to months to degrade completely, plastics take up to 500 years [1], making them a very harmful contaminant for the environment and the health of organisms. Their resilience is due to the complex unnatural chemical structure that they possess, for example, for polyethylene (PE), despite having ramifications that allow it to be first idiotically

*Author for Correspondence. E-mail: gabriel.salazar@metropoli.edu.mx

degraded, the subsequent biotic degradation by β -oxidation is hindered by lack of linear carbon chains; or in the case of polypropylene (PP), the presence of methyl groups in their hydrocarbon chains prevents their degradation by the same route [2]. The same happens with polystyrene (PS), polyvinylchloride (PVC) or polyethylene terephthalate (PET), which have very rigid and difficult to degrade chains [2]. It is for this and other reasons that alternative treatment options are currently being sought for this type of waste.

Currently, the method to recycle plastic waste is by mechanical recycling, which includes the collection, sorting, washing, and milling of waste. The main problem with this procedure is that many times, wastes of different types, sources and materials are mixed making this procedure very difficult, and that, under different conditions of temperature, oxidation, radiation, hydrolysis or mechanical breakdown, the different types of polymers degrade in an uncontrolled way. In addition to this, different polymers have different melting temperatures, which make their complete degradation even more complicated, and they end up being microplastics [3].

Micro plastics

Microplastics are fragments of plastics smaller than 5 millimeters [1]. They can be acquired through diet, and there is evidence showing that they can be transported by air and rain, making their inhalation possible, thus impacting human health even behind closed doors. The potential risk of microplastics in human health depends on their size and concentration, and they have been found in lung tissue, digestive system, circulatory system, urine, bile, cerebrospinal fluid and breast milk [4].

The potential risks to human health due to microplastics can be various, for example, they can cause blood vessel embolization, inflammation, genotoxicity, oxidative stress, apoptosis, and necrosis; as well as accumulate in tissues such as lymphoid nodules, liver or spleen, and for this reason, they can be a source of toxins and residual monomers that move into tissues and fluids within the body, which can turn carcinogenic. Microplastics may also alter the microbiota of the intestine and lungs [4].

Another concern is observed in treatment plants, for example, only in the USA or Europe, about 90% of microplastics are retained in activated sludge, that unfortunately, 50% of these sludges are used for agriculture, which inevitably brings them back to the daily food supply chains. Numbers are estimated between 63,000 to 430,000 and 44,000 to 300,000 tons of microplastics that reach farms in Europe or the United States respectively, which far exceeds the estimated 93,000 to 236,000 tons on the ocean surface [5]. It is for all these reasons that microplastics need to be completely degraded from the environment.

Plastic biodegradation

Due to the constant growth of plastic waste in the environment and the complexity to carry out its complete elimination when they turn into microplastics, biodegradation is perhaps one of the best options to address this problem. Biodegradation is the process by which organic substances are

broken down by living organisms, and among these, microorganisms are promising candidates to carry out this task by being spread throughout the environment [6].

According to Shah, Hasan, Hameed, and Ahmed, cited in [7], biodegradation involves several steps: biodeterioration, which is the combination of biotic and abiotic factors to fragment materials into smaller fractions; depolymerization, in which microorganisms secrete enzymes, like esterases or cutinases[8] or free radicals to break the polymers into oligomers, dimers, and monomers; assimilation, in which some molecules are recognized by cell receptors and are assimilated; and mineralization, when these molecules are oxidized to release simpler molecules such as CO₂, N₂, CH₄, H₂O, and other different salts. Biodegradation can occur by soil microorganisms as well as in aquatic environments [7].

In addition to degrading plastic, some research demonstrated that the isolated bacteria not only present degrading capabilities, but also, they are capable of changing the plastic's chemical structure or the polymer surface [9 & 10]. Even more, research demonstrated the appearance of aldehyde, ether, carboxyl and ketones groups when plastic was degraded by bacteria, which showed that it needs to modify the plastic prior to degradation [7]. This has also been demonstrated for fungi, one example showed that the plastic that got in contact with plastic degrading fungi lost 60% of its resistance, perhaps due to modified chemical structure [11]. Another work showed by scanning electron microscopy and atomic force that the surface of the plastic became rougher and deteriorated due to bacterial activity [12].

Also, the research may indicate that microorganisms are unable to break the polymer as it is, but instead, they need to transform it to molecules more amenable to be degraded [13], which indicate that they are not natural degraders of plastics and might need adaptations in their metabolisms to carry the degradation of the plastics chemical structure.

It also may indicate that microorganisms that degradation of plastic might produce other metabolites as by-product, which might or might not be harmful to other organisms or the surrounding environment. For example, the degradation of polyethylene (PE) releases volatile alkanes, which is apparently a normal byproduct that occurs during the degradation of this plastic [14].

Finally, the research shows that pretreatment of the plastic with certain agents might improve the degrading capabilities of the microorganisms. For example, PE that was pretreated with solutions of Tween 80, chlorine, and ethanol and then inoculated with bacteria showed that the degradation percentage was higher in comparison with other studies made with PE [15]. Also, the pretreatment of plastic with UV-light improved its biodegradation by a consortium of microorganisms from 15.8% (not pretreated) to 29.5% (pre-treated plastic), showing that the pretreated plastic was more prone to be degraded by microorganisms [16].

A summary of microorganisms that degrade plastic is presented in the following tables. Each shows the percentage of degradation in weight loss, according to the type of plastic and its conditions of growth. Table 1 shows plastic degrading bacteria isolated from soil, table 2 shows plastic degrading bacteria isolated from marine ecosystems, table 3 shows plastic degrading fungi

and table 4 shows different consortia of plastic degrading microorganisms. The information shows that there are a vast number of microorganisms that can biodegrade plastic.

Table 1. Bacteria isolated from soil that biodegrade plastic.

Genera and species	Origin	Plastic	Degradation (%)	Culture conditions	Reference
<i>Streptococcus</i> sp., <i>Pseudomonas</i> sp. and <i>Bacillus</i> sp	Chennai, India.	Low density PE	~23	Bacteria grown individually in Nutritious Broth with 2x2 plastic strips for 30 days.	[6]
<i>Bacillus subtilis</i>	Various soils of Mumbai, India.	PS	59	Bushnell Haas media during a month of incubation.	[17]
		PE	74.6	Nutritious Broth during a month of incubation.	
<i>Staphylococcus arletae</i>	Garden soil in India.	PE	9.09	Incubation at 30-37 °C for 30 days in mineral medium and with PE added to the medium at a concentration of 0.1%.	[9]
<i>Pseudomonas putida</i>	Soil of a municipal dump in Aurangabad, India.	PE	30	Synthetic culture medium with plastic discs as the only source of carbon, incubated for 30 days.	[10]
<i>Bacillus subtilis</i>			22		
<i>Bacillus amylolyticus</i>			20		
<i>Pseudomonas fluorescence</i>			16		
<i>Bacillus firmus</i>			12		
<i>Pseudomonas</i> sp.	Mangroves of the Vellar estuary of the southeast coast of India.	PE	20.54	Cultivation in Nutritious Broth with 1 cm discs, incubated for 30 days.	[18]
<i>Staphylococcus</i> sp.			16.39		
<i>Moraxella</i> sp.			7.75		
<i>Micrococcus</i> sp.			6.61		
<i>Streptococcus</i> sp.			2.19		
<i>Bacillus cereus</i> NBAlI B7	Flooring of a waste area of a plastic industry in	PE	17.39	PE strips grown in 50 mL of culture medium with washes and bacteria removal periodically for one month.	[19]

	Kolkata, India.	PVC	22.22	Cultivation in Petri dishes with Nutritive Agar, where PVC strips were placed and incubated at 30 °C for 45 days.	
<i>Bacillus</i> sp.	Various soils of Dehradun, India.	PE	42.5	Individually incubated in 3x3 cm plastic strips pretreated with Tween, chlorine and ethanol, grown in Nutrient Broth for up to 40 days.	[15]
<i>Staphylococcus</i> sp.			20		
<i>Pseudomonas</i> sp.			7.5		
<i>Bacillus cereus</i> A5,an (MG64264)	Ground of a Dandora dump in Nairobi, Kenya.	Low-density PE	35.72 ± 4.01	Incubation in synthetic medium with PE as the only carbon source for 16 weeks.	[7]
<i>Brevibacillus borstelensis</i> B2,2 (MG645267)			20.28 ± 2.3		

Table 2. Bacteria isolated from marine ecosystems that biodegrade plastic.

Genera and species	Origin	Plastic	Degradation (%)	Culture conditions	Reference
<i>Kocuria palustris</i> M16	Pelagic waters of the Arabian Sea in India.	Low-density PE	1	Incubation for 30 days with plastic as the only source of carbon.	[20]
<i>Bacillus pumilus</i> M27			1.5		
<i>Bacillus subtilis</i> H1584			1.75		
<i>Bacillus</i> sp. AIIW2	Marine ecosystem.	Low density PE	0.96 ± 0.02	Incubation for 90 days.	[12]
		High density PE	1.0 ± 0.01		
		PVC	0.26 ± 0.02		
<i>Bacillus</i> sp.	Coastal region of Tamil Nadu, India.	High-density PE	>25	Incubation for 30 days in a synthetic medium with plastic as the only source of carbon.	[21]
<i>Pseudomonas</i> sp.					

Table 3. Fungi that biodegrade plastic.

Genera and species	Origin	Plastic	Degradation (%)	Culture conditions	Reference
<i>Aspergillus</i> sp. and <i>Fusarium</i> sp.	Chennai, India.	Low-density PE	44	Fungus grown individually in Potato Dextrose agar as 2x2 plastic strips for 30 days.	[6]
<i>Aspergillus niger</i>	Various soils of Mumbai, India.	PE	52.9	Incubated in Rose Bengal medium for 30 days.	[17]
<i>Aspergillus glaucus</i>	Mangroves of the Vellar estuary of the southeast coast of India.	PE	28.8	Cultivation in Rose Bengal medium with 1 cm discs, incubated for 30 days.	[18]
<i>Aspergillus niger</i>			17.35		
<i>Aspergillus oryzae</i> A5,1 (MG779508)	Ground of a Dandora dump in Nairobi, Kenya.	Low-density PE	36.4 ± 5.53	Incubation in synthetic medium with PE as the only carbon source and added with Ampicillin for 16 weeks.	[7]
<i>Aspergillus fumigatus</i> B2,2 (MG779513)			24 ± 3.26		
<i>Aspergillus nidulans</i> E1,2 (MG779511)			18 ± 2.20		
<i>Aspergillus oryzae</i>	Contaminated plastic soil from Thanjavur, Tamil Nadu, in India.	PE	30	Incubation of the fungus for 60 days on the ground with plastic strips.	[22]
<i>Aspergillus niger</i>	Effluent soils contaminated with dyes from an area adjacent to the textile industries of Tiruppur, Tamil Nadu, India.	PE	38	Incubated for 60 days in culture using as a single carbon source 1 gram of PE strips.	[23]
<i>Aspergillus flavus</i>			31.2		
<i>Aspergillus foetidus</i>			26.1		

Table 4. Consortia of microorganisms that biodegrade plastic.

Genera and species	Origin	Plastic	Degradation (%)	Culture conditions	Reference
<i>Microbacterium wajiense</i> , <i>Rhodococcus jostii</i> , <i>Mycobacterium vanbaalenii</i> , <i>Streptomyces fulvissimus</i> , <i>Bacillus simplex</i> , and <i>Bacillus sp.</i>	<i>Lumbricus terrestris</i> worm intestine.	Low-density PE	60	Incubation for 30 days in low carbon soil.	[14]
<i>Lysinibacillus xylanilyticus</i> and <i>Aspergillus niger</i>	Floor of a landfill previously contaminated with plastic.	Low-density PE	29.5	Incubation for 4 months on artificially contaminated soil with plastic strips previously irradiated with UV light.	[16]
Bioaugmented consortia with <i>Lysinibacillus sp.</i> and <i>Salinibacterium sp.</i>	Coastal sites of Greece.	Low-density PE	19	Bioaugmented consortia of microorganisms previously adapted for plastic biodegradation.	[13]
<i>Nectriagliocladioides</i> , <i>Penicillium ochrochloron</i> and <i>Geomyces pannorum</i>	Commercial land	Polyester PU	60	Incubation for 44 days in commercial soil with temperature at 22 °C and controlled humidity.	[11]

Metabolism for the biodegradation of plastics

It is important to study the metabolism of microorganisms that carry out the biodegradation of plastic to understand and establish new bioremediation strategies. For this, a review was made that summarizes some of the studies done so far in the genus *Pseudomonas sp.* to elucidate different degradation mechanisms of various plastic wastes [24]. This genus is one of the most cited in research conducted on the biodegradation of plastics and could serve as a basis for future studies of the metabolism involved in plastic biodegradation.

Figure 1 shows a metabolic pathway for the degradation of PET for *Pseudomonas mendocina*, which describes the activity of an extracellular cutinase that acts on the polymer, producing the PET intermediates mono - (2-hydroxy-ethyl) -terephthalate (MHET) or bis 2-(hydroxyethyl) terephthalate (BHET) for later degradation to terephthalic acid (TPA) and ethylene glycol (EG),

which are later incorporated into the cell via a transporter for its degradation [24]. Then, the route for the degradation of isophthalic acid (IPA) and TPA by the bacterium *Comamonas testosteroni* was proposed as the route of degradation for *P. mendocina* [24&25]. In this, the induction of their degradation is given by two different dioxygenases followed by two dehydrogenations, for both routes to coincide in the formation of protocatechuic acid (PCA) which will then be metabolized by the cycle of Tricarboxylic Acids [24&25]. Ethylene glycol can be metabolized by the Glyoxylate Cycle or by other biosynthetic pathways (from Ronkvist et al., cited by Wilkes & Aristilde, 2017) [24].

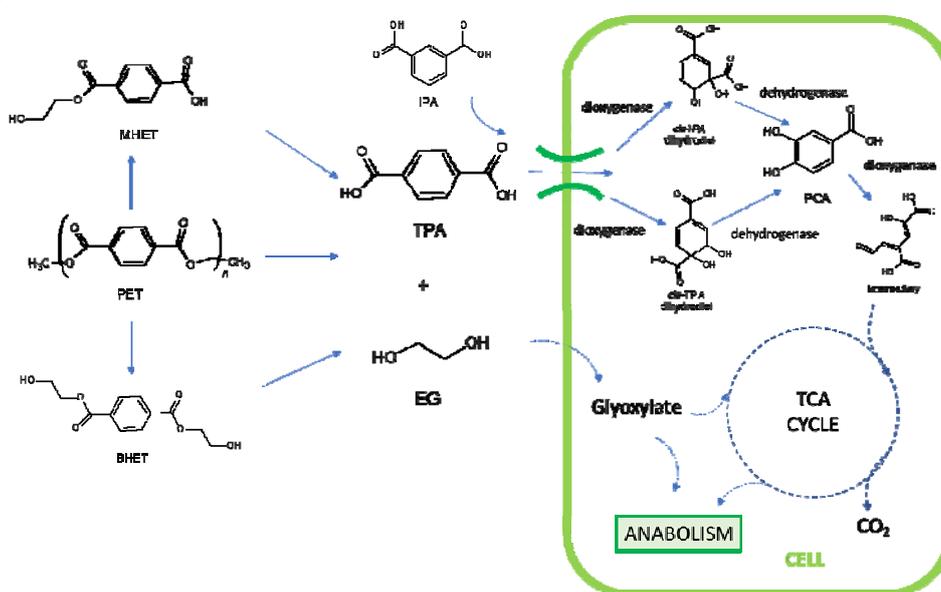


Figure 1. The metabolism of PET by *P. mendocina* is presented. An extracellular cutinase breaks the polymer to form the intermediates mono - (2-hydroxy-ethyl) -terephthalate (MHET) or bis 2-(hydroxyethyl) terephthalate (BHET), both of the monomers from PET are then hydrolyzed to ethylene glycol (EG) and terephthalate (TPA). TPA is internalized inside of the cell for subsequent metabolization to protocatechuic acid (PCA) to then enter the Tricarboxylic Acid cycle (TCA) for anabolism. It also works for isophthalate (IPA). EG is metabolized through the Glyoxylate bypass cycle or directly for anabolism. Dotted lines represent multiple metabolic steps (Adapted with permission from Wilkes & Aristilde, 2017 and Wang et al., 1995, [24 & 25]).

In the case of the degradation of PE, figure 2, a series of steps have been proposed that include the oxidation, dehydrogenation, and breaking of carbon bonds extracellularly to form acetic acid which enters the cell to go towards the cycle of Tricarboxylic Acids or other biosynthetic routes (from Lenz, cited by Wilkes & Aristilde, 2017) [24]. It is possible that the pathway is cycled until the complete degradation of the polymer is achieved.

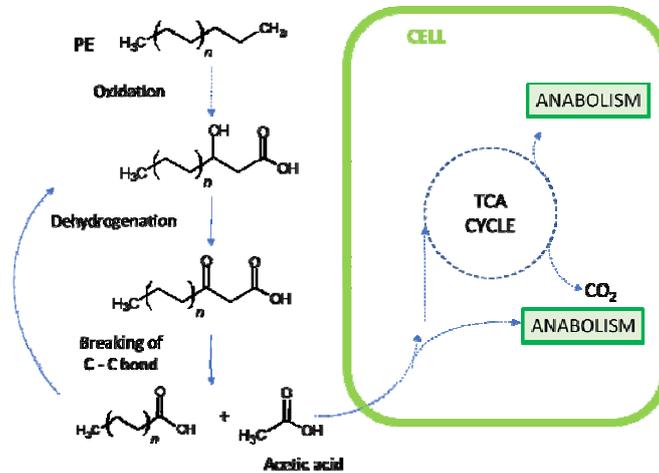


Figure 2. Proposed route of PE degradation. It suffers a series of oxidations and dehydrogenations in order to break a C – C bond to release acetic acid, which then can enter the TCA or directly go to anabolism. The dotted lines represent multiple metabolic (adapted with permission from Wilkes & Aristilde, 2017, [24]).

Another bacterium studied is *Comamonas acidovorans*, whose biodegradation activity of polyester-polyurethane (PU) was already compiled [26]. This bacterium synthesizes a membrane-bound esterase that catalyzes most of the degradation of polyester-PU. The degradation route proposed for the degradation of this plastic is presented in figure 3, during which secondary metabolites are produced to finally generate ammonium and diethylene glycol. This bacterium was capable of degrading polyester-PU completely after 7 days if it was used as the sole source of carbon [26].

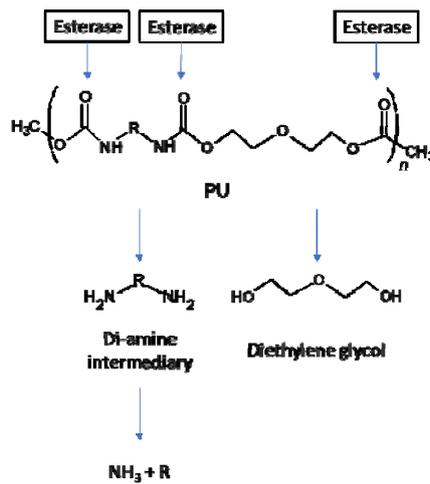


Figure 3. Degradation pathway by *Comamonas acidovorans* of polyester-polyurethane (PU). The polymer is attacked by esterases which break the carbonyl group to form diamine derivatives and diethylene glycol, to finally form ammonia (adapted with permission from Howard, 2011, [26]).

Finally, in the case of polystyrene, the proposal for its biodegradation includes its colonization and the secretion of enzymes that degrade it into smaller fragments [27]. Then, styrene alone can be used as a carbon source by bacteria like *Rhodococcus ruber* and *Brevibacillus* sp [27]. The most common is that styrene is degraded to phenylacetate and then enters the cycle of Tricarboxylic Acids as seen in figure 4. It must be mentioned that not all the styrene is used by the bacterium, since some of it accumulates inevitably as 2-vinylmuconate within the cell [28].

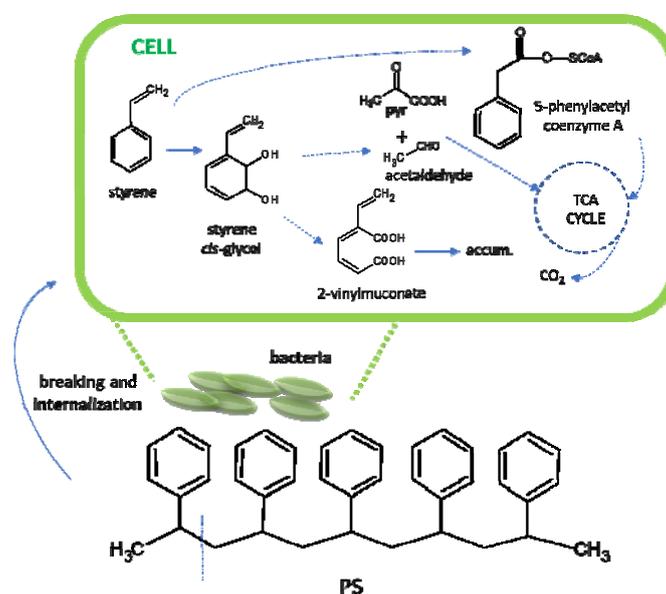


Figure 4. Degradation route for PS proposed in the 2018 review of Ho and collaborators. Bacteria colonize the polymer, and it breaks the monomer of the polymer to get its monomers. Then styrene is degraded through two different pathways to 5-phenyl-acetyl coenzyme A, pyruvate (Pyr) or acetaldehyde, which all of them may enter the TCA cycle. Another route can lead to 2-vinylmuconate, which accumulates within the cell (adapted with permission from Ho et al., 2018 and Mooney et al., [27 & 28]).

Genetics of plastic degradation

Different studies address the cultivation, identification and improvement of microorganisms for the degradation of microplastics, using genetic engineering as a tool for transforming various materials as alternative carbon sources through enzymes and proteins that allow successful biocatalysis. Among these studies, bacteria that stood out are *Pseudomonas pseudoalcaligenes*

and *Pseudomonas pelagia*, both were studied through a genome mining approach, from which cutinases and lipases were identified [30 & 31].

Many enzymes have been found to carry out the degradation of plastic by firstly degrading the polymers in their monomeric subunits, for example, many of the enzymes that degrade PET, which are hydrolases or related, perform the breaking of bonds by using water, releasing MHET or TPA, which are the building blocks of PET [32]. Among those enzymes, we find also cutinases, esterases and lipases, which increase the hydrophilicity of the polymer [33].

Also, enzymes with high performance on plastic degradation as MGS0156 had been isolated from *Desulfovibrio fructosivorans*, and more enzymes efforts had been reported in *Pseudomonas chlororaphis* (34, 35), *Pseudomonas protegens* (36) and *Pseudomonas putida* (37), for the degradation of polyurethanes (PU), with genetic identification of gene clusters encompassing seven open reading frames (ORFs) according to (36, 38 & 39). Also, similar enzymes have been identified in *Candida ethanolica* (40), and *Candida rugosa* (41), but the genetic assessment is not conclusive. The genome draft of an *Arthrobacter* sp. shows evidence of a nylon oligomer-degrading thanks to its essential enzymes for hydrolysis (30, 42, 43 & 44).

Some bacteria, like *Ideonella sakaiensis* strain 201-F6, can use PET as a major source of carbon and energy [45]. In an extensive work done by Austin et al. in 2018, they characterized the PETase from *I. sakaiensis* 201-F6 by means of X-ray crystallography and cloning experiments. They found out that it possesses a α/β hydrolase folding, which is common to cutinases and lipases, with a higher similarity to the cutinase of *Thermobifida fusca*. They found that the catalytic triad in the PETase of *I. sakaiensis* comprises the residues Ser160, Asp206 and His237, which reside on loops of the structure; also, Trp159 is a residue that extends the hydrophobic surface adjacent to its active site, which might favor its interaction with the polymer. Furthermore, they were able to produce a double mutant with a 4.13% increased enzymatic activity for PET degradation, these mutations were S238F/W159H. They also performed mutations in the Trp185 residue, finding out that it is an important residue which serves to accommodate aromatic interactions with the polymer [46].

Another experiment shed light about the relevance of the genes pueA and pueB for PU degradation in *Pseudomonas chlororaphis*. The degradation was more dependant of the pueA gene than in the pueB, and it is also important to note that both polyurethanases encoded by these genes comprise an active site formed by the triad Glu-His-Ser, in similarity with the PETase of *I. sakaiensis* [46 & 47]. Also, PETases and polyurethanases studied in different works showed a great degree of thermostability, by having a high residual activity at 90°C [32 & 47]. Phylogenetic analysis has revealed that polyurethanase enzymes have evolved from lipases, and from multiple lineages, which might explain their diversity [34].

It is interesting to note that the polyurethane esterase previously isolated from *Comamonas acidovorans* TB-35 by Nomura et al. in 1998 also has a catalytic site formed by the triad residues of Ser199-His433-Glu324, in which the glutamate residue replaces the most common aspartate residue [48]. This might indicate that this triad is a common trait in polymer degrading hydrolases. This enzyme also presents a hydrophobic domain near the active site, which in turn

might favor its interaction with the polymer, as was the case with the PETase of *I. sakaiensis* [46 & 48].

For the case of PE degradation, a hydroxylase translated from the alkane hydroxylase gene (alkB) of a *Pseudomonas* sp. strain E4 was reported, which degraded PE. This enzyme had a higher performance at degrading PE of low molecular weight than of high molecular weight, and showed high similarity with alkane hydrolases of other species of the *Pseudomonas* genera like *P. aeruginosa* or *P. mendocina* [49].

Bioinformatics have also played an important role for plastic biodegradation research, for example, Danso et al., in 2018 developed an algorithm for the identification of PET hydrolases, they identified over 800 potential candidates, and with all that information, they concluded that PET hydrolases occur in both marine and terrestrial habitats, but that this trait has yet not evolved enough to reach the degradation potential of enzymes that hydrolyze natural occurring polymers, like cellulose or starch. Also, in the same work, they identified that PET hydrolases originated mainly from three bacterial phyla, which are Actinobacteria, Proteobacteria and Bacteroidetes, the last one having the major amount of PET hydrolases in the marine environment. This is a rare finding which might turn the research focus towards the yet not well characterized phylum of Bacteroidetes [32].

Finally, in the wake of the plastic contamination problem, a new tool was developed by Gan & Zhang in 2019, which is a manually curated online database named Plastics Microbial Biodegradation Database (PMBD) that comprises more than 900 microorganisms, 8000 predicted enzyme sequences and 79 confirmed genes involved in the biodegradation of plastic. It includes biodegradation information for common plastics like PET, PE or PU, among others, and for not so common plastics, like poly-3-hydroxybutyrate (PHB), polylactic acid (PLA), butyl-benzyl phthalate (BBP), polybutylene succinate (PBS), and many others. Its link to its website is: <http://pmbd.genome-mining.cn/home>. Summarized information about some of the confirmed genes found to degrade PET, PE and PU, with the corresponding GenBank access number, is presented in Table 5; much of its information was obtained from the PMBD database [50].

Table 5. Genes involved in plastic degradation.

Gene	Enzyme	Microorganism	Plastic	Gen Bank accession	Ref.
cut1	Cutinase 1	<i>Thermobifida fusca</i> DSM44342	PET	HQ147787.1	[33]
cut2	Cutinase 2	<i>Thermobifida cellulositica</i> DSM44535	PET	HQ147786.1	[51]
est1 and est2	Esterase	<i>Thermobifida alba</i> AHK119	PET	AB445476.2	[51]
FOQG_13916	Cutinase 3	<i>Fusarium oxysporum</i> f.	PET	JH658423.1	[52]

*		sp. raphani 54005			
ISF6_RS23955*	PETase from the diene lactone hydrolase family protein	<i>Ideonellasa kaiensis</i> strain 201-F6	PET	NZ_BBYR01000074.1	[45]
cut190	Cutinase from the alpha/beta hydrolase family protein	<i>Saccharomonos poraviridis</i> AHK190	PET	AB728484.1	[53]
cut-2.KW3	Cutinase	<i>Thermobifida fusca</i>	PET	FR727681.1	[32]
TCUR_RS06300*	Triacylglycerol lipase	<i>Thermomonos poracurvata</i> DSM 43183	PET	NC_013510.1	[32]
cut1*	Cutinase 1	<i>Thermobifida alba</i> DSM:43185	PET	HQ147784.1	[32]
lipA	Lipase PET5	<i>Oleispira antarctica</i> RB-8	PET	FO203512.1	[32]
AAW51_RS12360*	PET12 of the diene lactone hydrolase family protein	<i>Polyangium brachysporum</i> strain DSM 7029	PET	NZ_CP011371.1	[32]
alkB	Alkanemone oxygenase AlkB	<i>Paenibacillus</i> sp. strain DK1	PE	MK045309.1	[54]
pudA	Polyurethane esterase	<i>Commamonas acidovorans</i> TB-35	PU	AB009606.1	[48]
pueA and pueB	Polyurethanase A and polyurethanase B	<i>Pseudomonas chlororaphis</i>	PU	EF175556.1	[34 & 47]
pueA	Polyurethanase esterase A	<i>Pseudomonas chlororaphis</i>	PU	AF069748.1	[55]

*Locus tag

Final remarks:

It is necessary to go deeper into the study on the capacity of microorganisms for the degradation of plastic, from finding more degrading species to how to potentiate the degradation performed by them by metabolism manipulation. For this, it is essential to carry out a thorough and detailed research.

It has been shown that there are many species of microorganisms that have acquired or are in the process of adapting for the degradation of plastic in the environmental, but it is not yet possible to develop technology with this information to obtain a benefit since the biodegradation of plastic is

still slow, incomplete and still greatly unknown. In addition, in natural environments where the microorganism is not isolated from other carbon sources, it is difficult for it to opt for the degradation of the synthetic polymer over a more accessible source, for this, more field tests are needed to measure these parameters.

Soils from different places are suitable for finding plastic degrading microorganisms, from mangroves to gardens and dumps, among many others. The most common species of bacteria are from the genera *Bacillus* sp. and *Pseudomonas* sp. and of fungi are of the genus *Aspergillus* sp. Soil bacteria degrade plastic in a greater percentage compared to marine bacteria and fungi better than both, even so, microorganisms in consortium perform better than when left in isolation. Something to note is that microorganisms can degrade the polymer before it becomes microplastic and thus, prevents it from undetectable damage to the organisms that inhabit the area.

The evidence shown here indicates that microorganisms first adapt for the degradation of plastic, from polymers into simpler molecules, starting extracellularly, to then carry out the internalization of their subunits and use them as carbon sources, making it difficult for them to achieve full degradation. But it is viable to facilitate this process by making the structure of plastics simpler by means of pretreatment with UV light, temperatures or acid or alcoholic solutions so that the polymer is more accessible to be biodegraded. This is the case of oxoplastics which are more readily degraded by microorganisms [29].

Bioremediation is a technology that is booming and has a lot of potential for minimizing the damage that anthropological activity has done to the environment, but more serious research is still needed on its weak points and the potential that can be exploited from them. Probably, for a more complete approach for the removal of plastic, a mix of several techniques must be put together in order to fully degrade this type of contaminant. But since this waste is usually found mixed with other types of contaminants, it may be necessary to adapt a broader array of technologies that must encompass the complete removal of the whole waste, which should include bioremediation of plastic waste by plastic degrading microorganisms. Kind of a similar way as a water treatment facility functions, with several stages aimed for removing different kinds of contaminants.

Also, the genetic engineering of plastic degrading microorganisms might enhance its degrading ability by overcoming the adapting step which natural microorganisms must undertake in order to degrade plastic. But dealing with GMOs in open environments might have several ethical and societal issues, which in turn might require an isolated facility, where the waste is moved in for further treatment.

The more information surfaces about plastic degrading enzymes and their genes, the closer will a plastic degrading biotechnology will be at hand. Every day, the characteristics that must be looked for in a plastic degrading enzyme are becoming clearer, which in turn, will make a GMO with industrial applications more feasible for soil or water treatment. More insight is needed to finally develop technology that will take advantage of the genetic and enzymatic power of microorganisms for plastic biodegradation, and it will become a topic of investigation for researchers in the near future.

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