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BIODEGRADATION OF PLASTIC POLLUTANTS AND IDENTIFICATION OF MICROORGANISMS CAPABLE OF DEGRADING PLASTIC POLLUTANTS: REVIEW

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Abstract

Plastic can be a good solution for the industry and, even if plastic products might be designed and produced for circular usage, conventional methods production of it and waste management are not reliable solutions anymore.
Plastic pollution became a very serious problem for the environment around the world today. Several studies shown us that microplastic is a very dangerous form of pollution and increasing amounts of it can be found in animals and human's bodies. However, recent studies provide us important data about the involvement of microorganisms in biodegradation of polymer materials.
Biodegradation process is based on the ability of some microorganisms to degrade certain plastic pollutants through their metabolic activity. Many microorganisms have the ability to secrete specific degradation enzymes that participates in degrading processes of plastic.
In this review we discuss about various microorganisms and their role in plastic degradation. We review different types of approaches and applications of molecular biology used for identifying microorganisms capable of degrading polymers and key genes involved in polymers degradation.

Key words*: biodegradation, pollution, microorganism, polymers, molecular biology.*

INTRODUCTION

Although, in the last decades, new plastic products have been introduced to the market, claiming to be better for the environment, lately plastic waste poorly managed turned into a very serious threat: plastic pollution, which became a real problem for the environment and human health (Bahl et al., 2020). Numerous studies reveal that due to the massive pollution with plastic wastes, microplastic particles in increasingly large quantities are found in humans and animal's bodies (Zhang et al., 2020).
With the increasing accumulation of plastic pollutants, modern society is facing a serious environmental global problem (Ali et al., 2021). The effects of plastic pollution started to became important threats for the well-being of marine and earth environment, but also for human health too (Emmanuel-Akerele et al., 2022).
The reason for the massive accumulation of plastic on agricultural land and the ocean is the low rate of degradation of artificial polymer products (Emmanuel-Akerele et al., 2022).
Annually, over 380 million metric tons (MMT) of polymeric products are manufactured, with a significant portion of approximately 10 MMT contaminating water bodies and oceans (Edwards et al., 2022). It was estimated that by the year of 2010, 275 MMT of plastic wastes have been generated and between 4.8 and 12.7 MMT of it ended up in the ocean (Jambeck et al., 2015). Now is estimated that around 80% of the plastic ever created will be discarded in the nature and that's because half of the amount of produced plastic is designed for single use (Edwards et al., 2022).
Plastic products are mainly produced from polyethylene terephthalate (PET), polyvinylchloride (PVC), polyethylene (PE), polystyrene (PS), polypropylene (PP), polyurethane (PUR) and polyamide (PA) (Edwards et al., 2022). Recent data suggested that more than 500 billion tons of PE is produced every year to fulfil the global request (Mohanan et. al., 2020).
When plastic waste is discarded into rivers, it ultimately makes its way to the oceans, resulting

in the destruction of the ecosystem (Fleming et al., 2014; Rachmawati et al., 2021). Scientists worldwide have reported that 267 species from aquatic environments are impacted by plastic waste (Ru et al., 2020).

It is known that plastic waste is decomposed in nature by oxidation, abrasion, sunlight and/or sunlight, but these natural-occurring processes take around 100 up to 500 years to completely decompose the wastes and, during this process, microplastic is formed (Law and Thompson, 2014; Rachmawati et al., 2021). Hence, it can be concluded that the slow rate of plastic decomposition and the formation of microplastic may result in toxic particle ingestion by both land and aquatic animals, as well as humans thorough the food chain (Ru et al., 2020).

According to De Tender et al. (2017) study, because of the slow degrading rate of plastic, plastic wastes can serve as a perfect media for bacterial colonisation. Also, another study conducted by Harrison et al. (2014) shown that colonisation on plastic wastes with microorganisms is faster than on other surfaces and the colonizing communities are taxonomically distinct from those in the unpolluted ecosystems (Zettler et al., 2013).

In 2012 Yoon et al. isolated several species of microorganisms like *Moritella*, *Pseudomonas* sp., *Streptomyces* sp., *Bacillus* sp., *Staphylococcus* sp., *Shewanella*, *Psychrobacter* capable of degrading plastic. Furthermore, numerous studies isolated and shown that fungus like *Aurebasidium pullulans*, *Fusarium solani, Curvularia senegalensis* and *Aspergillus* sp. are capable of using plastic waste as a sole carbon source (Sivan, 2011; Pramila and Ramesh, 2011; Usha et al., 2011; Rachmawati et al., 2021).

Biodegradation of plastic wastes is very complicated because of the unpredictable behaviour of microorganisms and it depends on many factors like morphology or surface characteristics and also molecular weight of the polymers (Mohanan et al., 2020; Ru et al., 2020).

As mentioned earlier, the process of polymer biodegradation is complex, and the type of plastic pays a significant role in determining its biodegradability.

Several studies have indicated that *Phormidium lucidum* and *Oscillatoria subbrevis* cyanobacteria, as well as *Galleria mellonella* and *Achroia grisella* waxworms, and bacterial strains such as *Enterobacter asburiae* and *Bacillus* sp., are capable of breaking down polyethylene (PE) (Mohanan et al., 2020; Saeed et al., 2022). Metabolic activity of these microorganisms plays a key role in biodegradation by secreting polyester-degrading enzymes (Shinozaki et al., 2013; Sriyapai et al., 2018; Mohanan et al., 2020).

Due to the many variables involved in biodegradation, including changes in the chemical structure of polymers, carbon dioxide emissions and substrate weight loss, recent studies and experiments have been focused on examining microbial activity, as well as isolating and identifying microorganisms that are capable of degrading plastic (Mohanan et al., 2020).

POLYETHYLENE BIODEGRADATION FOR SUISTANABLE WASTE MANAGEMENT

Polyethylene (PE) is a polymer composed of long-chain ethylene monomers (Emmanuel-Akerele et al., 2022) and has an average molecular weight of 300,000 Daltons (Da). Due to this high molecular weight, the microbial degradation rate of PE has been found to be approximately 0.26% to 0.29% after a period of two years. However, pre-treating PE with chemical oxidizing agents, thermos-oxidation and UV light can enhance its biodegradation rate (Mohanan et al., 2020).

Efforts in research have been directed towards discovering microbial strains that have the ability to break down and degrade PE. These strains have been isolated from various environments, including soil, seawater, compost, active sludge and even the gut of insects such as the wax worm (Nowak et al., 2011; Kyaw et al., 2012; Gajendiran et al., 2016; Sen and Raut, 2016; Montazer et al., 2020; Mohanan et al., 2020). Some species of bacteria, such as *Bacillus spp*. (Mohanrasu et al., 2015; Abrusci et al., 2013), *Rhodococcus spp*. (Bonhomme et al., 2003; Gilan et al., 2004; Fontanella et al., 2020) and *Pseudomonas* spp. (Rajandas et al., 2012), as well as fungi like *Aspergillus* (Hasan et al., 2007; Sahebnazar et al., 2010) and *Fusarium*, have been demonstrated the ability to break down PE after

undergoing certain pre-treatments, such as UV and/or thermal treatments (Mohanan et al., 2020).

Several bacterial strains, such as Pseudomonas putida IRN22, *Acinetobacter pittii* IRN19 and *Micrococcus luteus* IRN20, have been found to degrade untreated PE. In addition, different species of *Pseudomonas*, *Comamonas*, *Delftia* and *Stenotrophomonas* have also demonstrated the ability to degrade PE (Kyaw et al., 2012; Yoon et al., 2022; Peixoto et al., 2017; Mohanan et al., 2020).

Emmanuel-Akerele et al., isolated several microorganisms from soil in a study conducted in 2022. Soil samples containing plastic and plastic materials were taken from a dumpsite and studied over six weeks in agar culture and broth under laboratory conditions and their capability of degrading PE was analysed by the weight loss determination method (Emmanuel-Akerele et al., 2022).

To identify microorganisms, present in the sample, Emmanuel-Akerele et al. (2022) used methods described in Bergey's Manual of Systemic Bacteriology, namely Gram staining reaction, biochemical tests like citrate, mannitol test, oxidase, glucose fermentation and coagulase test. A number of 38 microorganisms have been identified, but from all microorganisms identified from the samples, only *Staphylococcus aureus*, *Streptococcus* sp., *Bacillus* sp. and *Micrococcus* sp. shown significant results regarding plastic degradation with the following rates: 25%, 31.2%, 25% and 31.2% during the four to six weeks period of time (Emmanuel-Akerele et al., 2022). In order to determine the degradation rate weight loss method was used. Results shown that the most efficient microorganism with the ability of degrading plastic after 6 weeks was *Staphylococcus aureus* closely followed by *Proteus* sp., *Streptococcus* sp. and *Bacillus* sp. (Emmanuel-Akerele et al., 2022). *Staphylococcus aureus*, *Pseudomonas sp*., *Bacillus sp*. and *Micrococcus sp*. had the highest degradation rate in different types of plastic and plastic waste having significant weight loss percentages, as it follows: 27.7%, 25%, 14.2% and 25% (Emmanuel-Akerele et al., 2022).

In another study from 2022, Saeed et al. aimed to isolate and identify bacteria and fungi that can degrade PE from soil samples collected from dumping sites in Pakistan. After a preliminary screening of microbial isolates, they identified four strains (two bacterial and two fungal) that showed promising biodegradation properties (Saeed et al., 2022). Molecular methods, including 18s rRNA sequencing, were used to identify the strains mentioned. The bacterial strains were found to be *Bacillus licheniformis* and *Achromobacter xylosoxidans*, while the fungal strains were identified as *Aspergillus niger* and *Aspergillus glaucus* (Saeed et al., 2022). A phylogenetic analysis was conducted on MEGA7 using the Neighbour-joining method. Based on their biodegradation screening results, selected strain was chosen for further experimental studies (Saeed et al., 2022). Saeed et al. research demonstrates that these microorganisms can significantly degrade PE leading to a reduction in the polymer's mechanical strength and stability. The biodegradation was confirmed through various techniques, including scanning electron microscopy and Fourier-transform infrared spectroscopy (FTIR). The study highlights the potential of these microorganisms in the degradation of plastic both in vitro and in soil burial methods.

In 2021, Rachmawati et al. conducted a study that aimed to isolate bacteria capable of degrading PE waste from costal ecosystem of Marina Beach in Semarang. Their study aimed to contribute to the understanding of the microbial community involved in plastic waste degradation in the ecosystem and potentially provide new candidates for bioremediation strategies (Rachmawati et al., 2021).

It is interesting to note that each ecosystem has its own unique bacterial diversity, as evidenced by the variations in the numbers and appearances of the different colony morphologies (Rachmawati et al., 2021). The mangrove ecosystem had the highest number if isolates with a diverse range of colony forms and colours, while the costal sand and rock ecosystem had the fewer isolates with similar colour appearances (Rachmawati et al., 2021). This suggests that each ecosystem may harbour distinct bacterial communities with different abilities to degrade plastic waste.

It is promising that eight out of the twelve isolates were able to form bright zones around the colony in the presence of PE and Congo Red

(Rachmawati et al., 2021). The presence of a bright zone surrounding bacterial colonies suggests that the studies isolates have the ability to decompose PE (Rachmawati et al., 2021). The size of the bright zone varied among the isolates, with 3 (three) of them showing the largest brighter zone diameter (Rachmawati et al., 2021). This suggests that these three isolates have a higher ability to utilize PE as a carbon source compared with other isolates (Rachmawati et al., 2021).

Raziyafathima et al. (2016) reported that *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri* have been found to degrade PE. Furthermore, it has been reported that *Enterobacteriaceae*, including *Enterobacter cloacae* and *Escherichia coli*, are capable of breaking down PE (Urbanke et al., 2018). Borowski et al. (2019) also documented that *Moraxella* species can degrade PE. Based on the results of Rachmawati et al. (2021) study, as well as the findings of Urbanek et al. (2018) and Borowski et al. (2019), it could be inferred that the isolates obtained from the Marina Beach region possibly belong to *Pseudomonas*, *Enterobacteriaceae* and *Moraxella* species.

In a recent study from 2023, Nademo et al. isolated and screened bacteria for their ability to degrade low-density polyethylene (LDPE) bags using both clear zone and weight loss methods. The most efficient isolates, KS35, KS14 and KS19 were further analysed (Nademo et al., 2023). Molecular identification of the isolates was carried out by 16s rRNA sequencing and the results showed that KS35 had 99% similarity with *Methylobacterium radiotolerans* MN525302, KS119 had 100% similarity with *Methylobacterium fujisawaense* KT720189 and KS14 had 99% similarity with species of *Lysinibacillus fusiformis* (Nademo et al., 2023). Their study suggested that these isolates have a high potential for degrading LDPE bags, which is significant given the global plastic problem.

BIODEGRADING POLYESTER FOR SUSTAINABILITY

In additional to microbial biodegradation, other methods have been explored for the disposal of polystyrene (PS) waste. These include chemical recycling and physical recycling. The process of chemical recycling entails the decomposition of PS into its constituent monomer, styrene, which can be then utilized to create fresh plastic goods. In contrast, physical recycling involves the melting and reprocessing of PS waste to manufacture new products. Both methods have their limitations and challenges, including high energy requirements and the generation of hazardous by-products. Therefore, microbial biodegradation remains a promising approach for the sustainable disposal of PS waste.

Studies have demonstrated that various microbes and microbial enzymes are capable of biodegrading PS, whether is pure PS, modified PS or PS blended with other polymers such as PLA or starch (Mohana et al., 2020). It has also been observed that the biodegradation rate of PS can be improved by blending it with other polymers. Specifically, *Pseudomonas aeruginosa*, *Curvularia species* and *Rhodococcus ruber* have shown the ability to degrade PS in various forms (Shimpi et al., 2012; Motta et al., 2009; Mohanan et al., 2020). Moreover, *Pseudomonas putida* CA-3 has been found to utilize styrene oil as its sole source of carbon and energy to produce PHAs (Ward et al., 2006; Mohanan et al., 2020).

In the same study from 2022, Emmanuel-Akerele et al. used the identified microorganisms to observe their degrading capacity on PS samples. After four, respectively six weeks it was observed that *Staphylococcus* sp. had the highest degrading ability after six weeks, while *Pseudomonas* sp. and *Lactobacillus* sp. did not degrade PS (Emmanuel-Akerele, 2022).

Jiang et al. (2021) reported the isolation of a PS-degrading bacterium identified as *Massilia sp*. FS1903 from the gut of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae that were fed with PS foam. *Galleria mellonella*, a common agricultural pest, has been found to have the ability to biodegrade PE and PS and this phenomenon is likely related to the gut microorganisms of these insects (Yang et al., 2015; Peng et al., 2019; Yang et al., 2020; Jiang et al., 2021). Prior research has established the existence of microbes in the gut of G. *mellonella* larvae that are involved in the degradation of PE, with the identification of the PE-degrading *Enterobacter* sp. D1 strain (Bombelli et al., 2017; Ren et al., 2019; Cassone et al., 2020; Jiang et al., 2021). However, studies focusing on PS-degrading bacteria from the gut of G.

mellonella larvae are relatively scarce. In Jiang et al. (2021) investigation, a PS-degrading bacterium was successfully isolated from the larval gut and identified through a combination of phylogenetic analysis and physiological and biochemical indicators. The study involved feeding *G. mellonella* larvae with PS foam for 21 days, after which their intestinal tissue was employed as a bacterial inoculum to enrich PS-degrading bacteria.

The intestinal cell suspension was placed into a flask with MSM and PS film and shaken for 60 days (Jiang et al., 2021). The remaining PS film was removed and dispersed the resulting enrichment culture onto LB agar plates, which led to isolation of pure bacterial strains (Jiang et al., 2021). During the logarithmic growth stage, genomic DNA was extracted from the bacterial cells using a commercially available kit (Jiang et al., 2021). Universal primers targeting the 16s rRNA gene were used for PCR amplification, and the resulting amplicons were visualized using agarose gel electrophoresis (Jiang et al., 2021). Finally, a phylogenetic tree was constructed using MEGA 5.0 software and Neighbour-joining method, with bootstrap values calculated from 1,000 replications (Jiang et al., 2021).

Based on its physiological and biochemical characteristics, the strains were identified as similar to microorganisms in the genus *Massilia*, which are commonly found in the rumen or large intestine of both humans and animals (Jiang et al., 2021). Phylogenetic analysis of the 16s rRNA gene showed that the tested strains FS1903 and *Massilia suwonensis* 5414s-25 had a high sequence similarity of 79% (Jiang et al., 2021). Considering the results of the physiological and biochemical tests, the strain has been deposited in the GenBank database under the accession number MW138062 (Jiang et al., 2021).

Jiang et al. (2021) study was the first study reporting the identification of a PS-degrading bacterial strain, FS1903, isolated from the gut of *G. mellonella* larvae. Further investigations are also necessary to assess the ability of the larvae to degrade other common plastic types, including PE, PP, PVS and PET, and to identify the underlying mechanisms and pathways responsible for this biodegradation.

EXPLORING THE BIODEGRADATION OF PVC

While there have been some reports of microbial consortia being able to biodegrade PVC materials, the research progress on PVC biodegradation is not as advanced as that for PE (Yang et al., 2014; Restrepo-Florez et al., 2014; Kumar Sen et al., 2016; Montazer et al., 2020; Ru et al., 2020), PET (Yoshida et al., 2016; Tournier et al., 2020; Ma et al., 2018; Edwards et al., 2022) and PS (Peng et al., 2019; Brandon et al., 2018). The degradation of PVC is more difficult compared to other plastics because PVC lacks a hydrolysable ester bond (Zhang et al., 2022). This suggests that there is a significant knowledge gap in understanding the microorganisms and mechanisms involved in PVC biodegradation (Peng et al., 2020; Giacomucci et al., 2019; Zhang et al., 2022).

Zhang et al. (2022) conducted an experiment to investigate whether *Spodoptera frugiperda* larvae could survive solely on PVC film and whether the larval intestinal microbiota could aid in PVC film digestion. Their study led to the isolation of *Klebsiella* strain EMBL-1, which was found to be capable of using PVC films as a sole source of energy and carbon. This finding is significant as it represents the first report of a bacterium capable of degrading PVC, and provides a potential avenue for developing bioremediation strategies to address PVC pollution (Zhang et al., 2022).

Zhang et al., in 2022, conducted an experiment, in triplicate, to verify the discovery that larvae of *S. frugiperda* can consume PVC film for survival. They compared the key physiological indexes (survival rate and body weight) and intestinal microbiota among larvae under three different conditions: starvation, feeding solely on PVC film and feeding normally on corn leaves (Zhang et al., 2022). After 5 days of cultivation, the survival rate of the larvae in the PVC group was found to be significantly higher compared to the starvation group, but still lower than the corn group (Zhang et al., 2022). The body weight of larvae groups followed the order: starvation < PVC < corn, indicating that PVC film can provide energy and sustain the survival of the larvae, although the growth efficiency on this specialized feeding on PVC films was lower than that with normal feeding on corn leaves (Zhang et al., 2022). To further

test their hypothesis that the intestinal microbiota is essential for PVC film degradation by *S. frugiperda* larva, Zhang et al. set up an antibiotic group in which gentamicin was used to inactivate most intestinal microbes of the larvae. Based on scanning electron microscopy (SEM) analysis, they found that PVC fragments recovered from excretion products in the PVC group showed a strong surface damage in contrast with antibiotic group, revealing the importance of intestinal microbiota for PVC degradation. These results shown the importance of the intestinal microbiota in the PVC biodegradation process by *S. frugiperda* larvae.

PVC film degradation by intestinal microbiota of *S. frugiperda* laervae is likely to create new ecological niches for microbiome selection through cross-feeding and this is because the degradation process leads to the release of transformation products (Zhang et al., 2022). (Zhang et al., 2022). The study by Zhang et al. (2022) showed that the degradation of PVC film by *Spodoptera frugiperda* larvae was accompanied by a significant shift in the composition of the larvae's intestine microbiota. Specially, the researchers found that the dominant phylum *Proteobacteria* was replaced bt a co-dominance of *Firmicutes* and that PVC feeding increased the diversity of bacterial amplicon sequence variants (ASVs) in the gut microbiota (Zhang et al., 2022). Certain bacteria such as *Enterococcus*, *Ochrobactrum*, *Falsochrobactrum*, *Microbacterium*, *Sphingobacterium* and *Klebsiella* were selectively enriched by the gut of microbiota upon feeding on PVC (Zhang et al., 2022). The study suggests that the intestinal microbiota of the larvae may pay a role in the degradation of PVC film (Zhang et al., 2022).

In the same study from 2022, Zhang et al. found that the larvae intestinal microbiota of *S. frugiperda* serves a significant source of PVC-degrading strains. Through laboratory screening, they identified a gram-negative strain called *Klebsiella* sp. EMBL-a, which formed a biofilm on the surface of the PVC film after 10 days of incubation (Zhang et al., 2022). Based on the given information, it can be inferred that the researchers observed visible cracks on the surface of the PVC film and an increase in biomass concentration, indicated by an OD600 rise from 0.20 to 0.60 (Zhang et al., 2022). This strain was identified thorough PCR cloning, sequencing and phylogenetic analysis of the 16s rRNA gene, and was found to be closely related to *Klebsiella variicola* and *Klebsiella pneumoniae* (Zhang et al., 2022). The researchers also discovered that the strain was able to alter the surface hydrophobicity and tensile strength of the PVC film, resulting in a weight loss of 19.57% after 90 days of incubation (Zhang et al., 2022).

According to the provided information, the use of advanced polymer chromatography (APC) indicated that the strain EMBL-1 was able to depolymerize the long-chain structure of PVC and generate fragments with lower molecular weight (Zhang et al., 2022). Furthermore, thermogravimetric analysis (TGA/DSC) demonstrated that the strain could attack the polymer chain of PVC and reduced its chemical stability (Zhang et al., 2022). These findings suggest that strain EMBL-a possesses the capacity to degrade PVC and modify its physical and chemical characteristics (Zhang et al., 2022).

The analysis of the strain EMBL-1's genome revealed that it belongs to the *Klebsiella* genus and has a closer relationship with *Kebsiella variicola* based on average nucleotide identity (ANI), which supports the findings from the phylogenetic analysis of the 16s rRNA gene (Zhang et al., 2022). The genomic analysis of strain EMBL-a revealed the presence of various genes that may be involved in the degradation of PVC (Zhang et al., 2022). With 5,646 predicted protein-coding genes, the strain's genome could provide valuable information about the biodegradation mechanism of PVC films (Zhang et al., 2022). These genes include those responsible for the degradation of aromatic compounds, as well as those involved in the metabolism of fatty acids and other organic compounds, indicating the strain's potential for PVC degradation and bioremediation purpose (Zhang et al., 2022).

The presence of these genes suggests that strain EMBL-1 is well adapted to degrading PVC, but further research is necessary to understand the underlying molecular mechanism responsible for PVC degradation by this strain.

RECENT ADVANCES IN PET BIODEGRADATION

Polyethylene terephthalate (PET) is an aliphatic polyester synthetized by the polycondensation reaction of monomers derived from the esterification of terephthalic acid and ethylene glycol, or the trans-esterification of ethylene glycol and dimethyl terephthalate. Due to its durability against solvents, impact, alcohols and moisture, PET is widely utilized in the packaging industry. Additionally, PET can be easily recycled, making it a popular choice for sustainability efforts.

In 2016, Yoshida et al. discovered *Ideonella sakaiensis*, a bacterium isolated from a plastic recycling plant in Japan, as part of ongoing efforts to develop more effective solutions for plastic pollution (Edwards et al., 2022). During their study, Yoshida et al. determined that *Ideonella sakaiensis* was able to use PET as a sole source of carbon along with other microorganisms. After their study it was concluded that using a bacterial consortium for biodegradation of polymers shows numerous benefits because of their mixed metabolism which can create a synergic effect in the process of degradation (Edwards et al., 2022).

In another study in 2020, Roberts et al. have been able to isolate, from soils contaminated with petroleum, a microbial consortium of *Psedomonas* and *Bacillus* sp. that was able to degrade PET in a synergic activity (Edwards et al., 2022). Previously, in a study from 2019, Leon-Zayas et al. revealed that microbial strains have "unique and diverse genome" (Edwards et al., 2022). Based on the study of Leon-Zayas et al. from 2019, a new study based on the synergy of microbial consortia, was conducted in 2022 by Edwards et al. trying to decipher the pangenome of microbial consortia and how, genetically and metabolically, PET can be degraded. Using the same type of microbial consortia isolated in 2019 by Robert et al. (*Pseudomonas* and *Bacillus* sp.), Edwards et al. analysed the pangenome gene cluster using MicroScope gene families (MICFAM) computed with the SiLiX software. During the analysis 232 core genomes from "all Pseudomonas and Bacillus were excluded and 259 different gene groups were found to be shared in the core genome with the pangenome of these five strains" (Edwards et al., 2022).

The study's findings revealed that *Bacillus albus* PFN01 strain 13.1 and Pseudomonas sp. B10 strain 9.2 had the most diverse collection of accessory genes compared to the other strain examined (Edwards et al., 2022). In particular, *Bacillus* strain 9.1 and 13.1 shared more than 3305 genes, while *Pseudomonas* strains shared over 22192 genes (Edwards et al., 2022). It was assumed that aldehyde dehydrogenases, esterase and alcohol dehydrogenases gene groups were involved in degradation of PET as they proved to be involved in PET monomer and oligomer's degradation (Edwards et al., 2022). Additionally, the study by Edwards et al. (2022) utilized RNA sequencing (RNAseq) to investigate the potential synergistic effect of microbial consortia. By comparing a consortium grown on L-asparagine as a control to one grown on PET, researchers observed an upregulation of genes associated with the initial degradation of PET, providing a possible explanation for the enhanced PET degradation observed in microbial consortia. Kumari et al. in 2021, after performing a transcriptomic analysis, concluded that aldehyde dehydrogenases found in Bacillus can be effective in PET degradation, "generating 4-[(2-hydroxyethoxy)carbonyl]benzoate from the deprotonation of free carboxy group of MHET".

During RNA extraction, an analysis comparing RNAseq transcripts from strain 10 and 13.2 revealed that TPA was being degraded (Edwards et al., 2022). The analysis also showed the presence of dioxygenases and decarboxylases that share similarity with phthalate oxireductases, such as 1.2-dioxygenase (Figure 10) (Edwards et al., 2022).

Both *Pseudomonas* strains 10 and 13.2 had upregulated genes related to PHA biosynthesis and carboxylesterase NlhH, which previously was identified in the pangenome as a potential PHA/PHB depolymerase (Jendrossek et al., 2002; Edwards et al., 2022). However, the deletion of NlhH in strain 9.2 did not show any reduction in np-butyrate hydrolysis, suggesting that there may be differences in esterase activity compared to EstB and NlhH may or may not be directly involved in PET polymer depolymerization (Edwards et al., 2022). Only strain 10 exhibited increased transcriptional levels of surfactin, and it had 113 upregulated

"hypothetical proteins", whereas only 77 were upregulated in strain 13.2 (Edwards et al., 2022). With the aid of pangenome of the complete consortium comprising three *Pseudomonas* and two *Bacillus* strains, Edwards et al. could anticipate which gene has the potential to degrade PET and identify many hydrolases, dehydrogenases and oxidoreductases.

After identifying EstB within pangenome, Edwards et al. proposed that this enzyme from *Pseudomonas* strain may be capable of hydrolysing PET/BHET. Edwards et al. compared the structure of IsPETase with the predicted structure of EstB and found significant similarities, including a binding cleft, catalytic triad and the absence of a lid structure, which suggests that EstB could be a PETase. Their observation of similar active sites and enzymatic activities, combined with a relatively low primary amino acid sequence identity between IsPETase and EstB, support the concept of convergent evolution in bacteria from different locations, allowing them to degrade PET and other plastics (Edwards et al., 2022). Additionally, the authors predicted that EstB could also have feruloyl esterase activity based on its alignment with the PMBD and other potential PET-degrading enzymes identified in the study (Edwards et al., 2022).

In 2021, Qi et al. tried an innovative approach for using artificial microbiota consortia to biodegrade PET or other types of polymers. In this study they created three artificial consortia to break down PET (Qi et al., 2021). Qi et al. (2021) genetically modified two strains of Bacillus subtilis to secrete PETase and MHTase, respectively. In addition, the included wild strain of Rhodococcus jostii and Pseudomonas putida to consume PET monomers TPA and EG (Qi et al., 2021). The researchers formed a four-species microbial consortium comprising Bs-PETase, Bs-MHETase, *R. jostii* and *P. putida*, which directly broke down PET into monomers and converted them into carbon dioxide and water through that tricarboxylic acid cycle (Qi et al., 2021). By doing so, they effectively improve the degradation rate by alleviating the metabolic inhibition of TPA and EG (Qi et al., 2021). This study presents a novel approach for using artificial microbial consortia to biodegrade PET and potentially other types of polymers in the future.

PU BIODEGRADATION

PU are commonly used plastic polymers that can be difficult to degrade. They are synthesized from polyols and polysiocyanates and can be classified into two types, polyester PUs and polyether PUs (Howard et al., 2000). Polyester PUs are more susceptible to microbial degradation (Pathirana and Seal, 1985; Howard et al., 2000).

Biodegradation of PU primarily occurs through hydrolytic cleavage of urethane bonds, but only a few microbial strains have been reported to efficiently degrade it (Nakajima-Kambe et al., 1999; Mohanan et al., 2020). While some fungal and bacterial species can degrade PU through enzymatic hydrolysis of ester linkages (Nakajima-Kambe et al., 1999; Howard, 2002; Mohanan et al., 2020), the efficiency of this process varies among different strains. Examples of fungal species that can degrade PU include *Aureobasidium pullulans*, *Cladosporium* sp., *Curvularia senegalensis* and *Fusarium solani*, while bacterial strains such as *Pseudomonas aeruginosa*, *Corynebacterium* sp., *Comamonas acidovorans*, *Pseudomonas fluorescens*, *Acinetobacter calcoaceticus* and *Bacillus subtilis* have been shown to use PU as a carbon, nitrogen and energy source for growth (Mohanan et al., 2020).

Cardenas Espinosa et al. conducted a study in 2020 to investigate the biodegradability of PU by a soil bacterium isolated from an area with brittle plastic waste. Through 16s rRNA gene sequencing and membrane fatty acid profile analysis, the strain was identified as *Pseudomonas* sp. (Cardenas Espinosa et al., 2020). It was found that the strain could use a PU-diol solution and a PU oligomer as the sole source of carbon and energy, and 2,4-diaminotiluene as the sole source of energy, carbon and nitrogen (Cardenas Espinosa et al., 2020). The researchers identified selected bacterial strains using the membrane fatty acid profile, while PLFA extraction was performed using the Blight and Dyer method, and identification and quantification of the fatty acid methyl esterase (FAME) were done using gas chromatography with flame ionization detector (GC-FID) (Cardenas Espinosa et al., 2020). Whole-genome sequencing revealed the presence of various catabolic genes for aromatic

compounds in the strain (Cardenas Espinosa et al., 2020).

For the genomic DNA extraction, the DNeasy R Blood & Tissue Kit from QIAGEN was used and the quantity of extracted DNA was checked by nanodrop (Cardenas Espinosa at el., 2020). The library preparation was performed with the NExtera XT DNA library kit, and the paired-end libraries were sequenced using Illumina v3 chemistry on Illumina MiSeq sequencer with a 250-bp paired-end protocol (Cardenas Espinosa et al., 2020).

To reveal similarities to known enzymes mono and dioxygenases, enzymes involved in aromatic degradation, amino acid sequences of genes present in the genome of TDA1 were compared to UniprotKB database or by using the basic local alignment search tool (BLAST) database in NCBI. The AROMADEG database was also used for the annotation of dioxygenases (Cardenas Espinosa et al., 2020). The proposed genes potentially involved in the degradation were identified through significant similarities in amino acid sequences, with high coverage and similarity and a low E value (Cardenas Espinosa et al., 2020).

In their study, Cardenas Espinosa et al. (2020) isolated two bacterial strains based on their growth rate on agar plates and in liquid media containing 2,4-TDA as the only carbon and energy source and was identified as *Pseudomonas sp*. TDA1. The isolated strain demonstrated the ability to utilize an oligomeric PU substrate and 2,4-TDA as a sole source of carbon, nitrogen and energy, along with other aromatic compounds, indicating its strong metabolic potential (Cardenas Espinosa et al., 2020). The identification of the strain was performed through analysis of its complete 16s rRNA gene sequences and phospholipid fatty acid profile, which resembled that of *Pseudomonas putida* KT2440, a *Pseudomonas* marker gene (Cardenas Espinosa et al., 2020). The findings support previous reports that *Pseudomonas* sp. is capable of degrading PU (Howard and Blake, 1998; Howard, 2002; Gautam et al., 2007; Hung et al., 2019; Cardenas Espinosa et al., 2020). Based on the genomic potential and substrate spectrum of the strain, the study proposes a degradation pathway for 2,4-TFA and identifies candidates genes encoding the enzymes involved (Cardenas Espinosa et al., 2020).

CONCLUSIONS

In recent years, the issue of plastic pollution has become a major concern, and finding effective and sustainable methods for plastic waste management has become urgent. Biodegradation of plastic waste is a complicated process that is affected by various factors, including morphology, surface characteristics and molecular weight of polymers (Mohanan et al., 2020). The microbial degradation of PE has been demonstrated to be feasible, with several bacterial strains such as *Bacillus spp*., *Rhodococcus* spp. and *Pseudomonal* spp., as well as fungi like *Aspergillus* and *Fusarium* showing potential for breaking down PE following specific pre-treatment methods (Mohanan et al., 2020). Microbial biodegradation, chemical recycling and physical recycling are some methods for the disposal of PS waste. However, microbial degradation of PS waste is a promising approach that can utilize PS waste as a carbon source and produce less harmful by-products compared to traditional recycling methods (Shimpi et al., 2012; Motta et al., 2009; Mohanan et al., 2020).

PVC is more challenging to biodegrade due its lack of hydrolysable ester bonds. Recent research has identified the gut microbiota of *Spodoptera frugiperda* larvae as playing a role in the digestion of PVC film (Zhang et al., 2022). Moreover, certain bacterial strains, including *Enterococcus*, *Ochrobactrum*, *Falsochrobactrum*, *Microbacterium*, *Sphingobacterium* and *Klebsiella* have been found to have the ability to degrade PVC (Zhang et al., 2022).

PP is highly resistant to biodegradation and while pre-treatment and blending with other materials can improve its biodegradability, recycling and reuse are more effective and sustainable solutions for PP disposal (Iwamoto and Tokiwa, 1994; Huang et al., 2005; Sameh et al., 2006; Mohanan et al., 2020).

Studies have used various techniques such as scanning electron microscopy and Fourier-transform infrared spectroscopy to confirm the biodegradation of plastic by microorganisms. The potential use of bacterial and fungal strains

in managing plastic waste highlights the potential of these microorganisms in the degradation process of plastic both in vitro and in soil burial methods and in the future could provide a framework for developing effective bioremediation strategies for plastic waste.

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