Microbial Degradation Alchemy of Plastics: An Overview of Bacteria and Fungi Responsible for Biodegradation of Plastics

Ana-Maria Căploiu[1], Teodor Vintilă[1], Nicolae Corcionivoschi[2] and Igori Balta[1*]

[1] *University of Life Sciences King Mihai I from Timisoara, 300645 Timisoara, Romania*
[2] *Bacteriology Branch, Veterinary Sciences Division, Agri-Food and Biosciences Institute, Belfast BT4 3SD, UK*

Abstract
Plastics have increasingly dominated our material landscape due to their widespread use. With the surge in the production and distribution of plastic products, the threat of global plastic pollution has escalated. This issue has increasingly alarmed environmental researchers due to the pronounced negative impacts on flora and fauna. As research efforts intensify worldwide, there is encouraging advancement in pinpointing and comprehending microorganisms capable of breaking down these plastics. The recent studies depicted microbial entities for their remarkable capabilities in tackling plastic waste. Among these are bacteria from the genera *Ideonella*, *Pseudomonas*, *Enterobacter* and *Brevibacillus*, as well as fungi, especially those from the genera *Aspergillus* and *Penicillium* which have showcased promising results in degrading plastics This scholarly review aims to clarify the lasting understanding concerning the biodegradation of polymeric materials. We delve into the different roles of specific microorganisms adept at plastic degradation, highlighting the enzymatic capabilities they employ. Additionally, we explore the environmental implications of leveraging microbial degradation as a sustainable approach to mitigating plastic pollution, offering insights into future research directions and applications.

Keywords: plastic biodegradation, polyethylene terephthalate (PET), plastic-decomposing microorganisms,

1. Introduction

Since its existence over a century ago, plastic has become quickly one of the most used materials by humans in order to create durable yet cheap items, ranging from simple things such as household items and shopping bags to key components used for cars and buildings. So, how did plastic become in such a quick timespan a human's "best friend?" It all started back around the early 20th century, Leo H. Baekeland was responsible for the discovery of "Bakelite" (a combination of phenol and formaldehyde), one of the very first types of plastic ever produced [1]. Plastic is a material composed of polymers, which are long chains of molecules called monomers bonded together. These monomers are primarily derived from hydrocarbons found in fossil fuels, such as oil or natural gas, making plastics either fully synthetic or semi-synthetic in nature. The specific properties of a plastic, including its durability, flexibility, and resistance to various environmental factors, are determined by the length and structure of its polymer chains. This variability allows for the creation of numerous types of plastics, each suited to different applications. For example, polyethylene is renowned for its versatility in packaging [2], polystyrene is widely used for insulation and protective packaging [3] due to its lightweight and insulating properties, and

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[*] Corresponding author: Igori Balta, Email: Balta.igori@usvt.ro

polyvinyl chloride (PVC) is used in construction for pipes and cable insulation because of its strength and durability [4]. Thus, the manufacturing of plastic has immensely increased and the "Plastic Age" we currently live in to this day has begun. Their lightweight yet durable nature offered a seemingly ideal alternative to traditional materials like metals and wood, enabling the production of a vast array of products at a fraction of the cost and weight. The initial promise of plastics led to an explosion in their popularity, which in turn spurred mass production on a scale previously unimaginable. From a relatively modest 30 million tons in 1988, global plastic production skyrocketed to an astonishing 359 million tons by 2018 and is estimated to raise by 590 million tons by 2050 [5, 6].

At the dawn of the 21st century, humanity faces an environmental challenge of unprecedented scale and complexity: plastic pollution. Synthesized for their durability, plastics have insinuated themselves into every facet of our daily lives, offering convenience and versatility. However, this very durability has become a double-edged sword, as plastics accumulate in oceans, landscapes, and even within the bodies of wildlife and humans, posing significant threats to ecological balance and health [7-9]. This pollution poses deadly risks to a wide range of marine life, including seals, fish, sea turtles, marine birds, sharks, whales, and filter feeders, among others. The consequences for these animals include entanglement, asphyxiation, and ingestion of plastic, which can result in internal injuries, digestive and reproductive issues, hormonal imbalances, and ultimately death. Additionally, plastic waste restricts the movement of marine animals and introduces toxins into their environment. Notably, plastic debris also serves as a raft for organisms like barnacles, algae, and mollusks, enabling them to, and even land animals such as ants and iguanas, to spread to new habitats [10]. This wide-reaching impact highlights the urgent need for addressing plastic pollution to protect marine biodiversity and prevent further ecological harm.

In response to this growing crisis, scientists and researchers have been exploring innovative solutions to mitigate the impact of plastic pollution. One of the most promising avenues of investigation is the field of microbial degradation. By harnessing the natural abilities of certain microorganisms to break down synthetic polymers, researchers are uncovering potential pathways to decompose plastics that were once thought to be indestructible biologically. Among the most promising avenues of research is the exploration of plastic degrading bacteria These microorganisms, equipped with specialized enzymatic machinery, possess the unique ability to break down polymeric chains of plastics into simpler, benign constituents *Ideonella sakaiensis* and others have been identified and characterized for their polyethylene terephthalate (degrading capabilities and metabolic pathways which reveal ed a potential biotechnological goldmine for developing effective biodegradation strategies [11].

As we stand at the ridge of a potential sustainable renaissance, this introductory review delves into the current knowledge surrounding plastic degrading bacteria It synthesizes findings from recent investigations, highlighting the mechanisms by which these microorganisms assimilate and degrade various plastics

2. Plastic-Degrading Bacteria: how it helps against plastic pollution

Plastic has been mainly degraded through abiotic ways such as photodegradation and thermooxidative degradation, but how would biotic degradation work? To do so, it would be easily with the help of microorganisms like bacteria, fungi and actinomycetes that are able to produce specific enzymes which catalyse hydrolysis reactions and form simple and less dangerous monomers. Plastics in the environment break down through one or more of four primary mechanisms: exposure to light (photodegradation), reaction with water (hydrolysis), breakdown due to heat and oxygen (thermooxidative degradation), and decomposition by living organisms (biodegradation) [12]. In natural settings like the ocean, the breakdown of prevalent plastics such as high density polyethylene (HDPE), low-density polyethylene (LDPE), and polypropylene (PP) typically starts with photodegradation, primarily triggered by UV-B radiation, followed by thermooxidation and, to a smaller degree, hydrolysis [12]. These processes result in the plastics breaking into smaller fragments and a reduction in the molecular weight of the polymers. Microorganisms can then digest these lower molecular weight compounds. During the biofragmentation phase, specific enzymes, including hydrolases and oxidoreductases, along with free radicals, contribute to the breakdown of the plastic's polymeric structure, further facilitating its decomposition. Unlike abiotic degradation of plastic (through mechanical, thermal, photo-oxidative and hydrolytic methods), biodegradation is much safer because less toxic substances are released from metabolic reactions and the obtained, simpler monomers can be re-taken by microorganisms to aid in their regeneration, growth and reproduction. Recent discoveries showed that there are many of potentially efficient plastic degrading microorganisms, ranging from bacteria to fungi which are exemplified in figure 1.

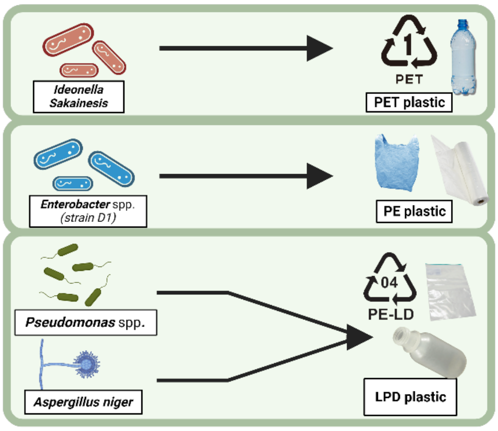

Figure 1. Microorganisms that degrade plastic. Created via Biorender.com.

3. *Ideonella Sakaiensis*

I. sakaiensis is one of the most used and praised microorganisms on this list regarding the degradation of plastics and microplastics thanks to its enzymes which were proven successfully to hydrolyze PET plastics from water bottles to much smaller components [13]. What makes PET plastic so resistant in comparison to other types of plastic is the chemical inertness terephthalic acid (TPA) has. TPA is a hydrophobic organic compound that is hardly degraded naturally in the environment, causing PET plastic in the end to be a problem when dumped in natural environments. But with the help of *I. sakaiensis s'* enzymatic processes, scientists can greatly reduce the dangers of plastic pollution. By looking through several run tests, *I sakaiensis* secretes 2 key enzymes: PETase and MHETase [14]. These enzymes have the ability to break down the complex nature of PET into much smaller monomers that could be re-used easier in other chemical cycles. PETase is the first key enzyme that acts on the decomposition of PET, resulting in a simpler compound, MHET (also known as Mono-2-hydroxyethyl terephthalate). The enzymatic reactions of PETase produced by this bacteria have a better hydrolysis effect at ambient temperatures (around 25°C) The second key enzyme, MHETase, directly hydrolyses MHET into 2 much simpler monomers: ethylene glycol and terephthalate [13, 14]. The simpler structures of the final resulting monomers can be easily recycled for other chemical cycles. Although this bacteria has been proven useful for breaking down PET plastic into simpler and less dangerous monomers, there are unfortunately some downsides that come around with these results. The bacteria has a better degrading efficiency mainly on amorphous PET (used for commercial food containers), but the metabolic actions are not as promising on highly crystalline PET (used in water bottles) under the same conditions. One way of helping *I. sakaiensis* to hydrolise highly crystalline PET is to melt down the plastic and rapidly cool it down [15]. An example of PET biodegradation via *I. sakainesis* is represented in figure 2 [16].

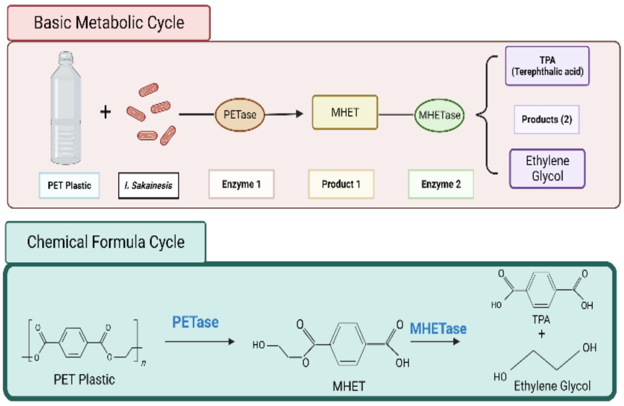

Figure 2. PET biodegradation via *Ideonella sakaiensis*. Created via Biorender.com.

4. *Enterobacter* Strain D1

Enterobacter is a genus of Gram-negative, anaerobic and rod-shaped bacteria abundantly found in water, sewage, inside guts and feces [6]. Although these microorganisms have earned titles as dangerous pathogens for often targeting urinary and respiratory tracts, there are several spp and strains that can be used by humans in plastic waste degrading industries. This intestinal bacterium

Enterobacter strain D1 found in the guts of *Galleria Mellonella* (Wax Moth) has shown abilities in some studies of its potential in degrading PE, one of the most used artificial polymers for material production and manufacturing [17]. The reason why wax moth larvae are used to screen the bacteria' metabolic activity is because their main diet consists of beeswax, which interestingly enough, has a very similar structure to PE plastic. In other words, Enterobacter sp D1 can break down PE plastic similarly to its activity on beeswax. The mechanistic insight of biodegradation of plastics by Wax moth is represented in the figure 3 [17]. Firstly, the larvae are immersed in ethanol for disinfection and the guts are removed. The guts are also washed with a sterile saline solution and then vortexed to obtain the intestinal homogenate used to screen *Enterobacter* D1. Other separation processes of centrifugation are used to separate the desired bacteria from the rest of the microflora to prevent biased results as much as possible. Once, *Enterobacter* has been successfully obtained, a small dish or tube with the appropriate medium is prepared. A thin and small PE film is also introduced, acting as the main carbon source for the bacteria. The dish/tube with the bacteria and plastic is then incubated for around 2-4 weeks at room temperature. At the end of the experiment, we should notice that the PE film the bacteria has degraded will present cracks and depressions observed via microscopy, therefore, we are aware metabolic activity has taken place [17].

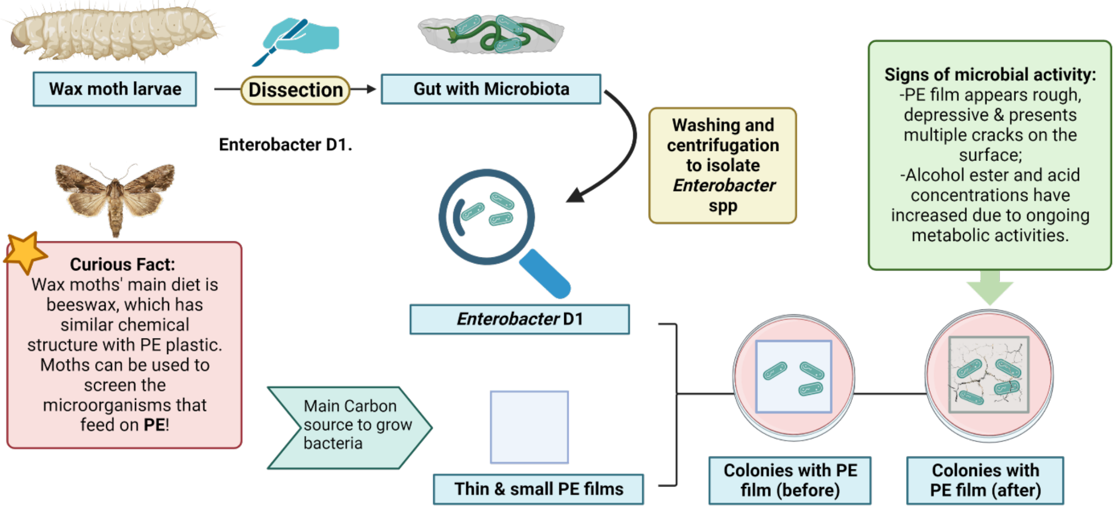

Figure 3. Experimental approach towards biodegradation of PE via *Galleria Mellonella* larvae purposed by Ren et al, 2019 [17]. Created via Biorender.com.

5. *Pseudomonas spp*

This is another *genus* of multiple Gram-negative, aerobic, rod-shaped bacteria that are abundant in the environment. They can be found in the air, water, soil and dead matter. For example, *Pseudomonas syringae* are also known to be nucleators of ice crystals in the clouds, making them responsible for snow and rain formation [18]. Many species of this genus are also able to resist to unfavorable environmental factors caused by high and low temperatures and different humidity conditions [19]. One of the most known spp from this *genus* is *Pseudomonas aeruginosa* because of its versatile roles human diseases have also taken advantage of in numerous industries such as medicine, agriculture, mining, oil refinery, textile making and waste decomposers [20].

Pseudomonas have a great metabolic capability of disintegrating multiple types of plastics, especially LDPE because of its effective enzyme secretions [21]. To harness bacteria for plastic degradation, microbiome samples from contaminated soil are collected, prioritizing those adapted to plastic environments due to their more efficient plastic-degrading enzymes. These bacteria are then isolated into a test tube containing inoculum, growth medium, and a thin LDPE strip as their primary carbon source. The test tube is weighed and its contents' concentrations noted, expecting a higher initial weight and greater substrate than biomass concentration [22]. After setting up, the test tube is incubated on a rotatory shaker at room temperature for a designated period. Post-incubation, the test tube is weighed again, with expected outcomes including a decrease in weight and substrate concentration relative to biomass, indicating successful degradation [22].

6. *Aspergillus niger*

Switching from the plastic-degrading bacteria to fungi eating bacteria, *Aspergillus niger* (black mold) is a great example of another microorganism that degrades PE plastic. Unlike some of the other microorganisms we talked about in this review, this fungus is much easier to grow and obtain as it isn't so strict to various environmental conditions. It fact, it is a very common fungus and can be found almost everywhere: from water and soil to decomposing organic matter such as feces or even fruit we left laying around in our house. *A. niger* is also a mesophile, meaning it favors growth in moderate temperatures with the lowest recorded temperature of 6°C and maximum 45°C [23], but in some cases, it can also resist to pretty high temperatures such as 60°C only for a limited period of time (around half an hour) [24]. This mold species is also xerophilic (grows in conditions with low humidity) but it also favors environments with 90%-100% humidity, therefore it is very flexible to growth in a diversity of environmental conditions. Therefore, A. niger's high flexibility of adapting to distinct environmental conditions makes it a great choice for the plastic-degrading experiments due to the fact that it's easier to work with the controls [22, 25]. So how does this mold help in plastic degradation? *A. niger* was used in multiple experiments of LDP-degradation with the same methods and series as *Pseudomonas* sp bacteria, with the main difference that *A. niger* had a better efficacy in plastic degradation than *Pseudomonas* spp alone, illustrated in the figure 4 [22]. However, the highest plastic-degrading efficiency results were noted when experiments used both the mold and bacteria to act on LDP at the same time. Although *A. niger* has offered a positive result in LDP degradation, there are unfortunately some risks in working with and cultivating this mold because of it pathogenic nature. Black mold is capable of producing toxins (e.g. OTA-ochratoxin A) that are opportunistic to affecting humans, especially to those who have a weakened immune system or allergic reactions to fungi.

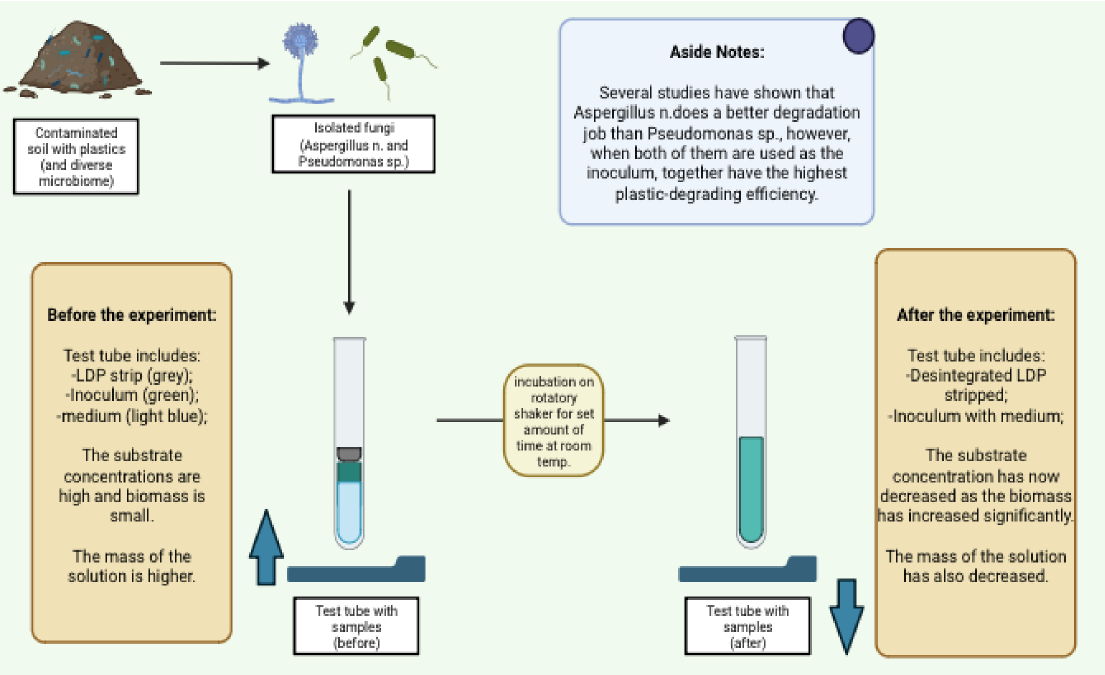

Figure 4. Representation of LDP Degradation with *Aspergillus niger* and *Pseudomonas* spp. Created via Biorender.com.

4. Conclusions

In conclusion, the recent discoveries and development of plastic-degrading microorganisms mark a significant step forward in our fight against plastic pollution. These microorganisms offer a promising biotechnological solution to the pressing environmental challenges Future research should focus on enhancing the efficacy of these bacteria and integrating them into holistic waste management systems. This strategy requires a collaborative approach, uniting the efforts of scientists, policymakers, and industry.

Of course, it is inevitable for cons to come along with their pros. For biodegradation, for example, the target plastics are now always fully or completely decomposed under abiotic factors for a long time, such as UV radiation, temperature and physical stress [13]. Also because of plastics' durable and complex nature, enzymatic processes take a longer period of time to break them down into simpler monomers. Another main disadvantage when working with plastic-degrading microorganisms is that some species are potential pathogens which can cause intoxications and various symptoms to humans who work alongside with them, so it is very important to take in consideration stricter health precautions during the experiments. [13]

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