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Review Article

Role of *Ideonella sakaiensis* in Reducing Plastic Waste: Current Updates and Future Prospects

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ABSTRACT

Plastic is a non-biodegradable material which does not leave the face of our planet earth. Its non-biodegradability and durability make it almost impossible to degrade biologically. On an average 1 million plastic drinking bottles are purchased per minute throughout the globe. Polyethylene terephthalate (PET) is one of the most widely used plastics which is used in the production of drinking bottles. Continuous efforts are being made to bring down the total plastic waste accumulation by either recycling or by chemical degradation, methanolysis, ammonolysis in laboratories, and in industries but these processes are not only time taking but are also cost ineffective and require a lot of labor and expertise in handling the chemicals. Alternatively, bio catalytic degradation can be applied as an eco-friendly alternative to degrade plastic. A bacteria named, *Ideonella sakaiensis* was reported that is capable of consuming and can breaking down plastic specifically, PET as an energy source and sole carbon. It consists of two enzymes which can degrade PET, PET hydrolase, and MHET hydrolase. It can prove as an excellent alternative for bioremediation of already accumulated plastic waste worldwide. Bacteria, *I. sakaiensis* could be considered as a great weapon in the efforts for sustainable development that are underway worldwide. The aim of our review is to highlight the importance of *I. sakaiensis* which can help in enzymatic plastic degradation.

Key words: Bacteria, Plastic accumulation, Polyethylene terephthalate, Non-biodegradable, Sustainability, Durability, *Ideonella sakaiensis*.

1. INTRODUCTION

Plastic is an important material in our day-to-day life routine due to the presence of desirable qualities such as durability, light weight, and low manufacturing price. With an annual productivity of about 300 million tons the total weight of plastic has increased enormously. Our addiction to, single use plastic has led to a large scale plastic production and hence landed us in major trouble by enormous plastic waste accumulation.

According to the known statistical analysis, only 9% of the total plastic ever produced is recycled till date [1].

More than 50% of the plastic production annually belongs to single use category. Studies indicate that almost 60–65% of the plastic produced in a year is either dumped in landfills or lands in oceans or the natural environment. If current practices continue to prevail, there will be more plastic than fishes in the ocean by 2050. The production rate of plastic has tripled over the last few decades and the graph of plastic waste has grown exponentially.

In 2015, a total of 5000 metric tons of plastic was dumped in landfills which sum up to a total of 12,000 metric tons by 2050, if the situation is not taken under surveillance [2]. Polyethylene terephthalate (PET) is the most used plastic since its development in the mid-1940s. The main properties which make it a better choice to be used on a large scale are its exceptionally great mechanical properties, thermal stability, impermeability for liquids and gases, and transparency. Applications of PET include use in textile fibers, in making of drinking bottles and food containers. The inert structure of PET is the main problem in its usage as PET is non-biodegradable in nature which makes it one of the most common components of accumulated plastic wastes.

In biodegradation of plastic, extracellular enzyme is secreted by microorganism, which gets attached to the surface of plastic. Enzymes hydrolyze plastic to its intermediate polymer. These polymers are assimilated by microbial cells to release CO_2 as carbon source.

In 2016, scientists [1] tested different bacteria from a bottle recycling plant and found that *Ideonella sakaiensis* 201-F6 strain could digest the plastic used to make single-use drinking bottles made up of PET material. It was first identified in 2016 by a group of researchers led by KOHEI ODA of Kyoto Institute of Technology and Kenji Miyamoto of Keio University JAPAN. The proposal of bacteria feeding on plastic (*I. sakaiensis*) in itself is a promising approach for the plastic crisis that desperately needs monitoring. The bacteria work by secreting an enzyme [3] (a type of protein that can speed up chemical reactions) known as PET hydrolase (PETase) which results in breaking of certain chemical bonds (esters) in PET, resulting in smaller molecules that the bacteria can absorb, using the carbon in them as a source for food. The super bacteria *I. sakaiensis* can significantly shorten the time of degradation of

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PET. The bacteria can be utilized on both the scales; small-scale or large-scale applications, such as domestic or industrial landfills, respectively [4].

2. BACKGROUND

PET also known as PET is the most extensively utilized plastic but it is leading to a lot of serious environmental hazards because of its structure being chemically dominant [5]. The conventional PET recycling methods absorbed a lot of energy resources and often generated hazardous by-products [6]. The utilization of enzymes obtained from microorganisms has given a novel and environmental-friendly alternative for establishment of biodegradation procedures [7]. PET hydrolyzing action has been observed in various carboxylic acid hydrolases such as lipase and cutinase [8]. Cutinase is one of the most thoroughly investigated PETase and is characterized for its high molecular weight polyesters developing capacity [9]. A lot of efforts have been made and protein altering schemes have been tried to increase the activity of cutinase [10]. However, alternatives with high efficiency and specificity are continuously being worked upon [11]. In recent times, a new PET degradation alternative has been reported from the bacteria *I. sakaiensis* isolated from PET bottle reprocessing plants in Japan [12]. Figure 1 here shows the mechanism of PET degrading into its monomers.

3. BIO-DEGRADATION OF WASTE PET

Monomers in PET are connected by ester bonds, which can be hydrolyzed by numerous hydrolytic catalysts found in nature. Evidently, the presence of hydrolytic enzymes makes PET more likely to undergo biological degradation than the polyolefin, as there are no known enzymes that could directly break the carbon-carbon bonds present in polyolefin.

Although many PET hydrolytic enzymes (PHEs) have been recognized, their ability to break down PET and its use as a carbon source by any microorganism has not been revealed. The main reason for non-biodegradability of polyolefin, PE, and other forms of plastics is due to weak or minimal reaction of carbon-carbon bonds present in their structure. Various other factors such as flexibility of the polymer chain and its crystallinity also affect enzymatic degradation.

Various researchers screened different microorganisms that degrade PET from the sample collected from various locations including PET debris from bottle recycling units. They expected various PHEs to be present in the samples collected. Eventually the efforts resulted in discovery of a microbial colony (201-F6 strain) that can grow on low crystallinity PET film [2]. The bacterial strain accumulated on the PET film and used PET as a significant carbon and energy source and degraded the film into CO_2 and water. Further studies lead to the confirmation of the presence of bacterial species that can metabolize amorphous PET without the help of any other organism present in the growing culture. The strain 201-F6 was named *I. sakaiensis*, from the genus *Ideonella.* Figure 2 here depicts different ways to deal with plastic pollution such as Bioremediation, through Special chemicals, through recycling of PET monomers, bio treatments, or through material recycling.

4. ROLE OF *I. SAKAIENSIS* IN PLASTIC DEGRADATION

The Gram negative, aerobic, rod-like bacterium from the genus *Ideonella* and family *Comamonadaceae* was able to degrade and consume PET as a sole carbon and source for energy generation. The bacteria lack Spore formation capacity. The optimum temperature requirements were 15–42°C and optimum pH of 5.5–9.0. Hence, it was given the name the plastic feeder bacteria [1]. After its initial degradation by *I. sakaiensis*, the microbial colony was observed to convert 75% of degraded PET into CO_2 [4].

5. ENZYMATIC CONSTITUENTS OF *I. SAKAIENSIS*

The bacteria, *I. sakaiensis* is contains two exclusive enzymes that catalyze hydrolysis of PET. PETase [4] separates PET into mono(2-hydroxyethyl) terephthalic acid (MHET), and MHET hydrolase (MHETase) separates MHET (mono hydroxyethyl terephthalate hydrolase) into terephthalic acid (TPA) and ethylene glycol (EG). In Figure 3, how PET is converted into TPA and EGA is shown through flowchart.

5.1. PETase

The sequencing of the genome of *I. sakaiensis* allowed the recognition of an enzyme with PET hydrolyzing activities [4]. It released a crater - like depression on the surface of an amorphous PET layer and liberated the hydrolysis by - product to the aqueous surrounding. On analysis of this enzyme with other known PHE's it was found that the enzyme found in the bacteria *I. sakaiensis* has the greatest catalytic activity for the PET degradation at optimum growth conditions [12]. It was classified as PETase.

A typical α/β-hydrolase fold is adopted by an enzyme which contains a twisted central β-sheet consisting of nine β-strands sandwiched by six α-helices. The structure of PETase shows the presence of two disulfide bridges.

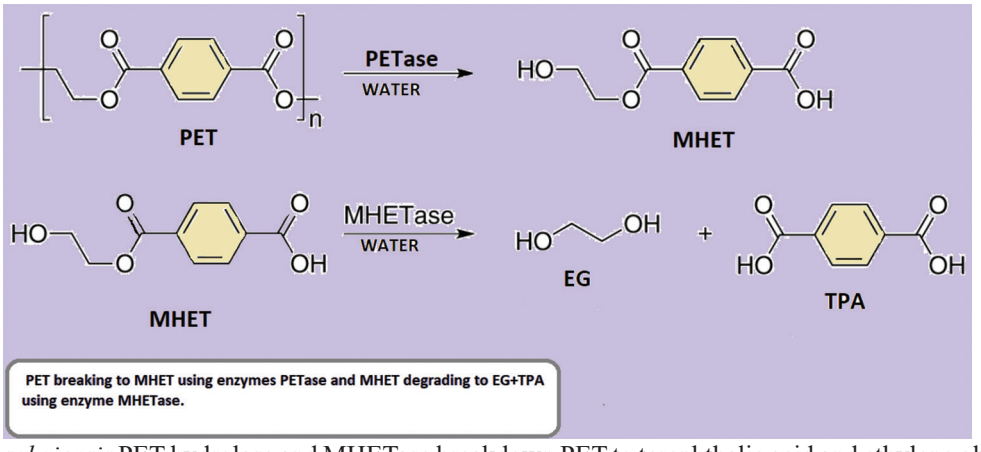

Figure 1: *Ideonella sakaiensis* PET hydrolase and MHETase breakdown PET to terephthalic acid and ethylene glycol. (EG-Ethylene glycol, TPA: Terephthalic acid).

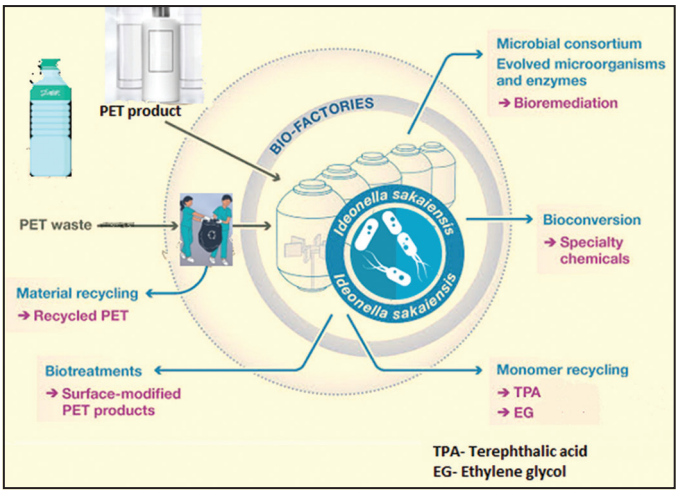

Figure 2: A sustainable way to deal with plastic pollution.

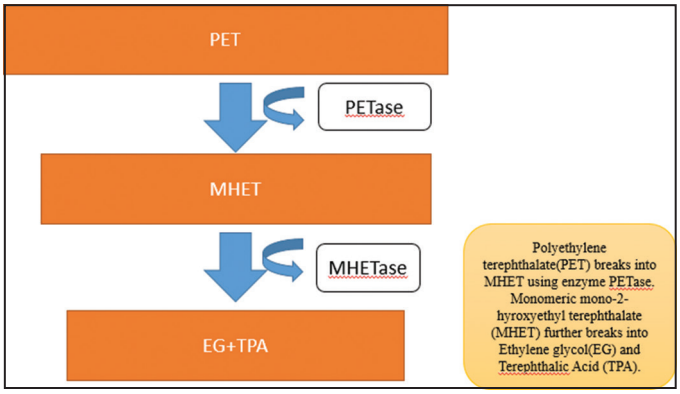

Figure 3: Flowchart depicting enzymatic action.

5.2. METase

I. sakaiensis contains another enzyme that is participating in PET metabolism [12] which has been tagged as a member of the tannase family. METase enzyme hydrolyzes (2hydroxyethyl) TPA (MHET), the main PET hydrolysis products of PETase, into TPA and EG. The structure consists of a α/β-hydrolase domain and a lid domain [13,14]. The α/β-hydrolase domain contains amino acid residues beneficial for catalysis, whereas the lid domain gives substrate specificity. The biochemical and structural analysis for

MHETase revealed that MHET binding to the catalytic pocket induces a structural change that blocks the active site, and the six amino acid residues that are found in the lid domain are in direct contact with the substrate that results in tighter binding and also in recognition of the correct substrate.

This rare combination of PHETase and MHETase and the arrangement of enzymes for degradation of PET is unique to *I. sakaiensis* and so far not been found in any other microbial organism with a genome that is fully sequenced.

6. POTENTIAL APPLICATIONS OF PETASE

The recognition of *I. sakaiensis* and its ability to absorb PET through hydrolysis, followed by downstream metabolism of its monomers, could provide a great solution for degradation of PET either using the bacteria directly or using enzymatic constituents present in the bacterial strain. The bacteria can contribute to sustainable development goals by being a part of genetic engineering.

Some of the possible approaches could be:

- The ability of the bacteria to remain stable in its composition even after repeated sub culturing could allow its usage directly in the environment for bioremediation since no special culturing, energy supply or media would be needed by the bacteria in the growth duration [14].
- The bacteria could be used to identify various genes that code for enzymes responsible for PET degradation and then in production of genetically modified microorganisms of desirable genes of interest and with genetically modified structure.
- PET-degrading microorganisms can be utilized to separate waste PET into its constituent units such as TPA and MHET for recycling and "bio-reusing" [15].
- PETase could be applied for the biodegradation of poly (ethylene furanoate) [15], which is an alternative for PET and a 100% biodegradable plastic material.
- Discovery of various other plastic degrading microorganisms can be done using the strategy that was used for screening *I. sakaiensis* 201-F6 strain.
- PET waste should be treated with *I. sakaiensis* to generate CO_2 that can be used as a fertilizer in sugarcane farms to enhance plant growth.
- Genetic modification can be carried out in the *I. sakaiensis* 201-F6 strain to recover the intermediate products TPA and MHET for resynthesis of PET [1].
- *I. sakaiensis* can be used for bioprocess production of PCA which is an important compound in the synthesis of value added chemicals such as 6,6 nylon, adipic acid, and catechol [16].

7. CONCLUSION

The pandemic led to a slowdown in even day to day activities but the graph of plastic accumulation kept on growing at an alarming rate even during lockdown. Plastic accumulation is a serious concern and needs immediate attention for a better future. The production of plastic started almost 50 years ago and it has already covered one third part of the planet by its accumulation. Alternatives for non-degradable plastic need to be discovered and measures should be taken to eliminate the already accumulated portion of plastic waste.

The discovery of *I. sakaiensis* provided valuable information by giving the knowledge of the presence of plastic degrading bacteria naturally in the ecosystem but the rate of degradation needs to be catalyzed to cope up with the problem of plastic accumulation on the surface of our planet earth. The bacteria can be genetically modified to work on a large scale for biodegradation of plastic waste that is already accumulated in the ecosystem. The bacteria can grow on low crystallinity PET and degrade it to liberate carbon dioxide and water but a lot more needs to be done since there are no possible ways for degradation of a lot of other forms of plastics. Use of science along with adequate use of microorganisms that can degrade plastic and other non-biodegradable materials can offer some adequate solutions to the alarming ecological issues due to plastic accumulation. More focus should be given to discovery of such microorganisms that can help in biodegradation of waste to speed up the process of bioremediation.

We can look up for alternatives of *I. sakaiensis* or genetically modify various microorganisms to cause hydrolysis of plastic in the environment. We can also look up for alternatives for our renewable resources and save our nonrenewable resources for the future generations to use. We can conclude that this newly discovered Bacterial strain of plastic degrading bacteria *I. sakaiensis* can be a great start in the processes of biocatalytic degradation in the area of bioremediation and sustainable development programs.

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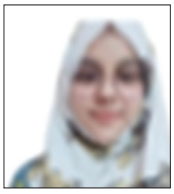
**Bibliographical Sketch*



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