Design and development of synthetic bacteria with built in genetic circuits for plastics biodegradation.

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Polyethylene Terephthalate (PET) is one of the majorly produced plastics which has a wide range of applications from household to industrial fields. The accumulation of PET plastics and related pollution in the environment have been global concerns for many years. The PET plastic production has increased to more than 500 billion tonnes in the year 2021. The discovery of PET degrading enzymes such as the PETase, etc. from a bacterium called *Ideonella sakaiensis* has opened new opportunities for many potential approaches to develop an efficient plastic biodegradation system. Out of many platforms available for this, we believe that synthetic biology approach can be the most effective way for the development of a novel system which is safe and efficient for the biodegradation of PET plastics.

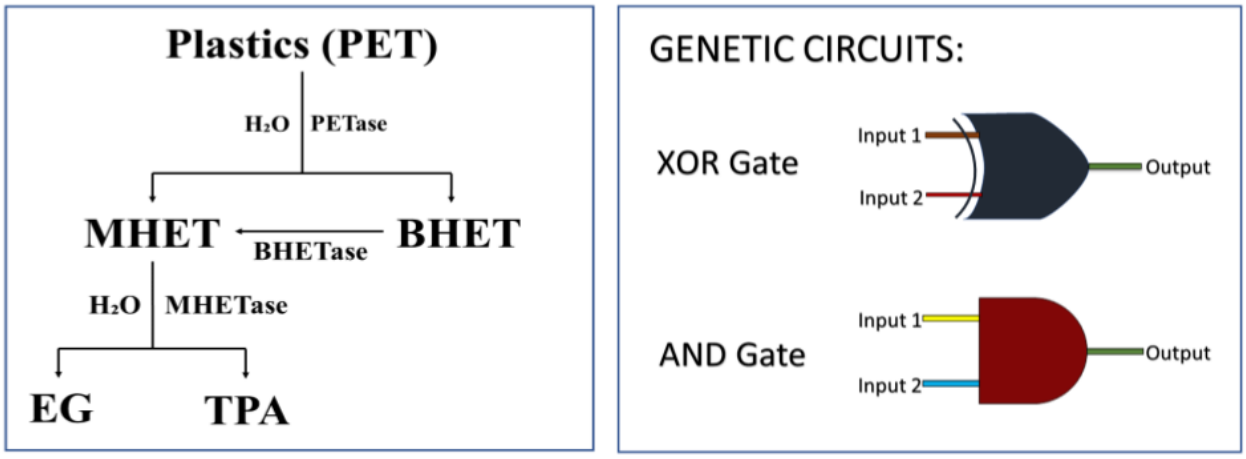

Figure 1. Flowchart of the pathway for PET degradation that occurs naturally in *Ideonella sakaiensis* (left panel) and the proposed genetic circuits for the synthetic bacterium (right panel).

Polyethylene terephthalate (PET) has been the biggest contributor of plastic waste where there has been only an increase in their production from 300 billion units in 2004 to 583.3 billion units in 2021 despite 29% of the PET plastics being recycled for their usage every year (1). PET is the major type of plastic which is used in the production of many household plastic items and in food and beverage packing items like plastic bottles, plastic boxes, etc. In the year 2016, a team of researchers in Japan isolated a bacterium called *Ideonella sakaiensis* from a plastic bottle recycling facility in Sakai, Japan (2). This bacterium was found to break down and metabolize plastic (PET) with the help of certain enzymes produced by it (2). *Ideonella sakaiensis* produces two main enzymes which have the capability to break down PET into its constituents. The two main enzymes are PETase and MHETase. PET being the primary substrate for the breakdown of plastic, the enzyme

PETase was found to breakdown PET into MHET (mono (2-hydroxyethyl) terephthalic acid) and BHET (*bis* (2-hydroxy) terephthalate) along with a small amount of TPA (Terephthalic acid) (2-4). Thus MHET, by the action of MHETase, produces TPA and ethylene glycol (2-4). BHET by the action of BHETase is converted to MHET which finally gives TPA and ethylene glycol as final products of this process of degradation (2-4).

Many genetic engineering approaches were applied for the enhancement of the enzymatic activity of the PETase and MHETase enzymes till date (5). The end products TPA and ethylene glycol being toxic in nature, along with the enhancement of the enzymatic activity of PETase, MHETase and BHETase, a system must be designed for their safe and effective application in the degradation of PET plastics. Synthetic Biology is an effective platform which involves the designing and development of novel organisms or redesigning the existing naturally present biological systems for useful purposes (6). To design a novel organism which has the capability to degrade plastics (PET), a Synthetic Biology approach was chosen. This approach involves the usage of genetic engineering to enhance the enzymatic activity and building of genetic circuits for designing the genome of the novel organism and then evaluation of the novel organisms for their activity. This approach is expected to give a better solution for the prevailing plastic pollution caused due to the accumulation of PET plastics.

Currently, we are in the process of analyzing the pathways involved in the degradation of plastics by *Ideonella sakaiensis* and designing of genetic circuits based on the analysis for the construction of the genome of the novel organism. Genetic circuits developed for the initial steps of reactions involved in the PET degradation are displayed in Fig. 2. The ongoing and upcoming research on building these genetic circuits for the construction of a novel organism will be published in the future issues of TCABSE-J.

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