Press Release

京都工芸繊維大学 KYOTO INSTITUTE OF TECHNOLOGY

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Kyoto Institute of Technology

Discovery of a Bacterium that Degrades and Assimilates Poly(ethylene terephthalate) could Serve as a Degradation and/or Fermentation Platform for Biological Recycling of PET Waste Products

A group of researchers comprising Keio University Faculty of Science and Technology Assistant Professor Shosuke Yoshida (currently a researcher for the ERATO Akiyoshi Bio-Nanotransporter Project at the Graduate School of Engineering, Kyoto University), Keio University Associate Professor Kenji Miyamoto, Kyoto Institute of Technology Professor Emeritus Kohei Oda, and Professor Emeritus Yoshiharu Kimura, conducted a collaborative research project with Teijin Limited and ADEKA Corporation and discovered a bacterium that degrades and assimilates poly(ethylene terephthalate) (PET) and were able to identify the decomposition mechanism.
PET is used extensively throughout the world, commonly in clothing and plastic bottles. Most PET products simply end up in landfills, never entering a recycling process, and it was generally believed that PET is resistant to microbial degradation.
However, the present research overturns certain aspects of this commonly accepted theory, and the findings are expected to contribute greatly to the development of bio-recycling technology of PET waste products.
The results were published in the American science journal, *Science*, on March 10, 2016 (Eastern Standard Time).

1. Main Research Findings

- Researchers have discovered a novel bacterium *Ideonella sakaiensis* 201-F6 that is able to degrade and assimilate PET.
- Researchers have discovered two types of enzymes* (PETase and MHETase), which are produced by the 201-F6 strain and are capable of hydrolyzing PET.
- Based on the properties of PETase and MHETase, it is now understood that the 201-F6 strain is able to use PET as its major energy source and exist in the natural environment.

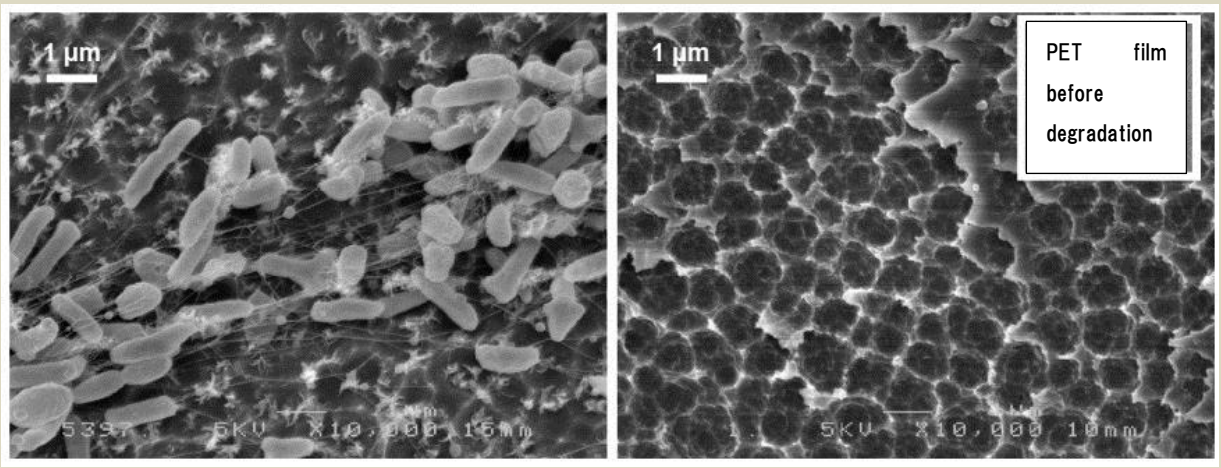
2. Research Background

PET is made from petroleum and is widely used in clothing and plastic bottles. About 56 million tons of PET was produced worldwide in 2013 alone, of which 15.4 million tons were for food and liquid containers, 3.2 million tons for packaging films, and 38 million tons for synthetic fibers.
Currently the only PET products being recycled are bottles, but the amount recycled accounts for just 37% of the total production volume of PET bottles (6.13 million tons), which is a mere 4.1% of the entire production volume of PET. Most PET waste products are disposed of as garbage and introduced into the environment. To create a healthy ecosystem and pursue sustainable development, society must break its dependency on limited resources and encourage a shift toward a recycling-based society. Currently the main method employed for recycling PET waste

uses chemical techniques, but these processes come with many problems, such as the vast amount of energy consumed.
PET is a very stable material and thus resistant to microbial degradation. However, identifying a microorganism that uses PET as its energy source, and by using its bio functions, we would be able to propose a low-energy, environmentally friendly strategy for biological recycling of PET waste products.

3. Research Content and Results

The research began with a search for microbial PET degraders in the natural environment. A variety of environmental samples were taken and cultivated in test tubes containing a defined culture medium supplemented with a PET film as the major carbon source. After a few weeks, one sediment sample contained a distinct microbial consortium which had formed on the PET film, and the film was showing clear signs of degradation. From the microbial consortium, we then successfully isolated the bacterium capable of degrading PET. As the bacterium was discovered from environmental samples taken in Sakai, Osaka Prefecture, it was given the name *Ideonella sakaiensis* 201-F6. We then discovered that this 201-F6 strain not only degrades but can also assimilate PET and support microbial growth.

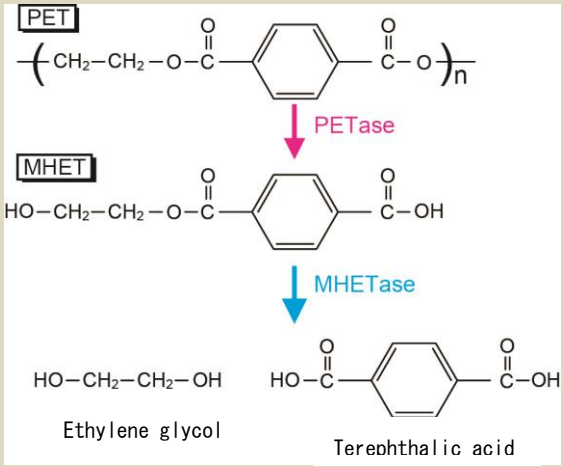


(Left) 201-F6 formation on PET film (Right) Degraded PET film surface after washing out adherent cells

We then tried to understand the degradation mechanism and conducted a genome† analysis to acquire more information on the enzymes that can break down the PET polymer. We identified genes that were similar in sequence and code to enzymes that have been known to hydrolyze†† PET. We then conducted a function analysis of the protein gene product, which revealed its ability to catalyze PET hydrolysis. Surprisingly, we found that, compared to previously known PET-degrading enzymes (whose fundamental function is thought to be the hydrolysis of other esters of high molecular weight), this newly identified enzyme had a higher preference for PET degradation and was more active, even in room temperatures where PET molecular structure is at its densest.It is possible that these abilities are acting as a sort of weapon for the 201-F6 strain to survive in the natural world using PET as its energy source. Taking this characteristic into account, we named this particular enzyme PETase.
Furthermore, we noted that, after the PETase enzyme broke down PET into the compound MHET (synthesized by the esterification of terephthalic acid and ethylene oxide), there was no

further occurrence of decomposition. Supposing the existence of a MHET hydrolytic enzyme, we conducted an exhaustive gene expression analysis¶, and identified an enzyme with very similar gene expression patterns to PETase. Through the function analysis of the protein encoded in this gene, we were able to find out its ability to swiftly hydrolyze MHET. Showing high affinity to MHET, the newly identified enzyme was termed MHETase.

From the above results, it is understood that the environmental 201-F6 strain bacterium uses two enzymes—PETase and MHETase—to efficiently break down PET into its two environmentally benign monomers, terephthalic acid and ethylene glycol, which are then broken down further to produce carbon dioxide and water. After this stage, it is reported that many other bacteria capable of degrading terephthalic acid and ethylene glycol have been found. Until now, PET has always been thought to be immune to biodegradation, thus continuing to keep filling our landfills, but the present research has revealed that there may be a biological solution to introducing PET in the cycles of matter.§



The *Ideonella sakaiensis* 201-F6 strain uses two types of enzymes to break down PET into monomers

4. Future Research

Compared with chemical processes, PET degradation using microorganisms and enzymes consumes less energy and is a more environmentally friendly option. We believe that the present research results bring us closer to achieving an ideal model for PET recycling, so long as we are able to enhance the activity level and stability of these newly discovered microbial enzymes.

About the Article

Title: A bacterium that degrades and assimilates poly(ethylene terephthalate)

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Terminology

* Enzyme: Enzymes are produced in living cells and are proteins that catalyze metabolic reactions.

† Genome: The genome consists of deoxyribonucleic acid (DNA) and is the genetic material of an organism. A bacterial genome contains a few thousand densely packed genes.

†† Hydrolysis: Hydrolysis of esters involves reaction with water molecules to produce acid and

alcohol.

¶ Gene expression analysis: Gene expression is the process by which information from a gene is converted into the structure and function of the cell. For the present research, messenger RNA, which takes on an intermediary role during this process, was extracted from cells and comprehensively studied using a next generation sequencer.

§ Cycle of matter: Cycle of matter refers to the process of synthesis and decomposition of matter within the natural environment.

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