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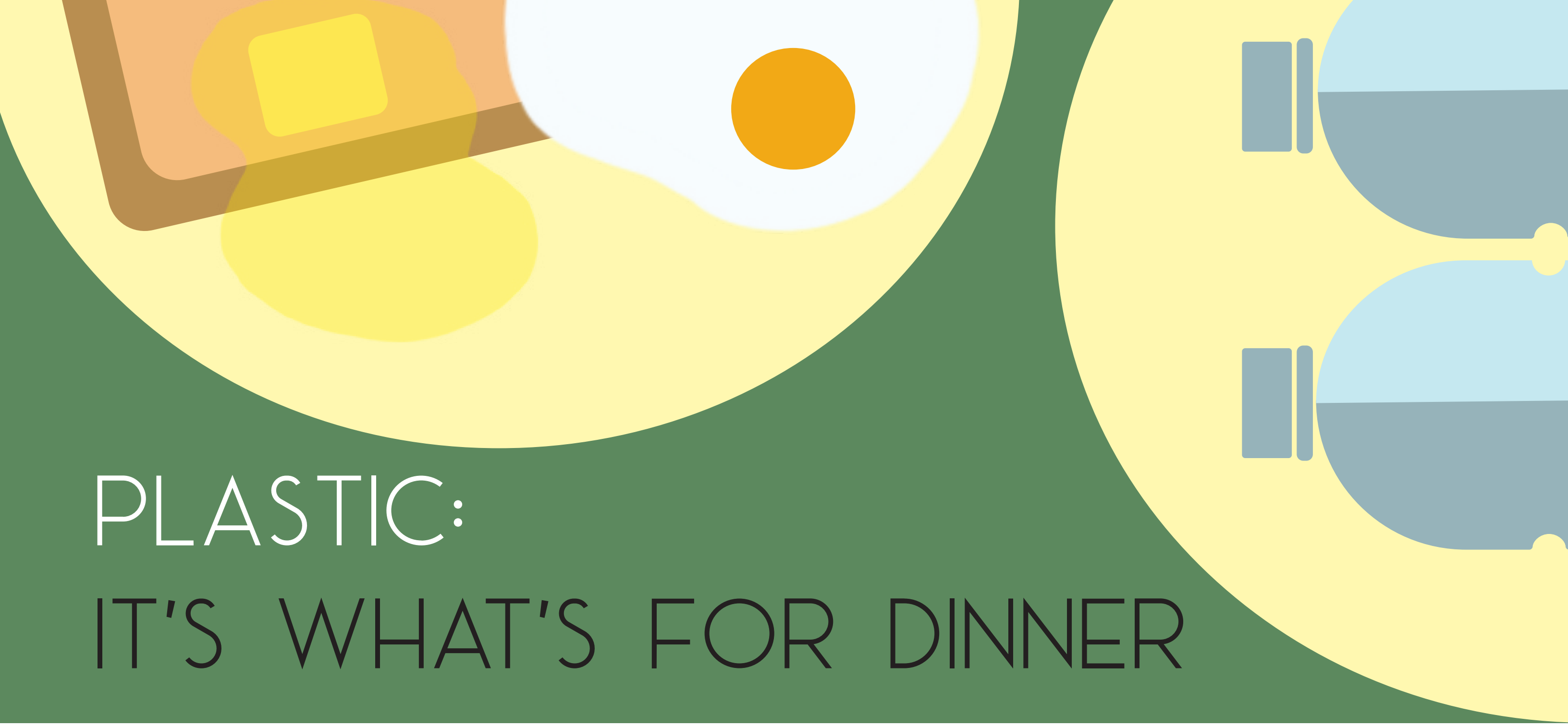

PLASTIC: IT'S WHAT'S FOR DINNER

BY EMILY PEARLMAN

When was the last time you used plastic? Was it the film over your microwaveable meal last night? A produce bag at the grocery store? A plastic water bottle? Plastic is everywhere, including many places it doesn't belong—tiny plastic particles have been detected in the oceans, the soil, and even the air.[1] It's no secret that plastic waste is a major global problem, one we will have to address soon if we want to prevent serious environmental destruction.

Enter *Ideonella sakaiensis*: a plastic-eating bacteria discovered in the soil outside of a bottle recycling facility in Sakai, Japan. Identified in 2016, *I. sakaiensis* is one of the few organisms that is able to use plastic as its main carbon and energy source.[2] Studying this bacteria provides insight into plastic biodegradation and the promise of innovative approaches to bioremediation and recycling.

So how does *I. sakaiensis* do it? Let's look at some of the key players. Polyethylene terephthalate (PET) is its meal of choice. PET is the most abundant polyester in the world, found in many common products like plastic bottles, packaging, and clothing.[3] Like all plastics, PET is a long, chainlike molecule called a polymer, which is made up of repeating units called monomers. You can think of the monomers as beads which, when strung together, form a polymer necklace.

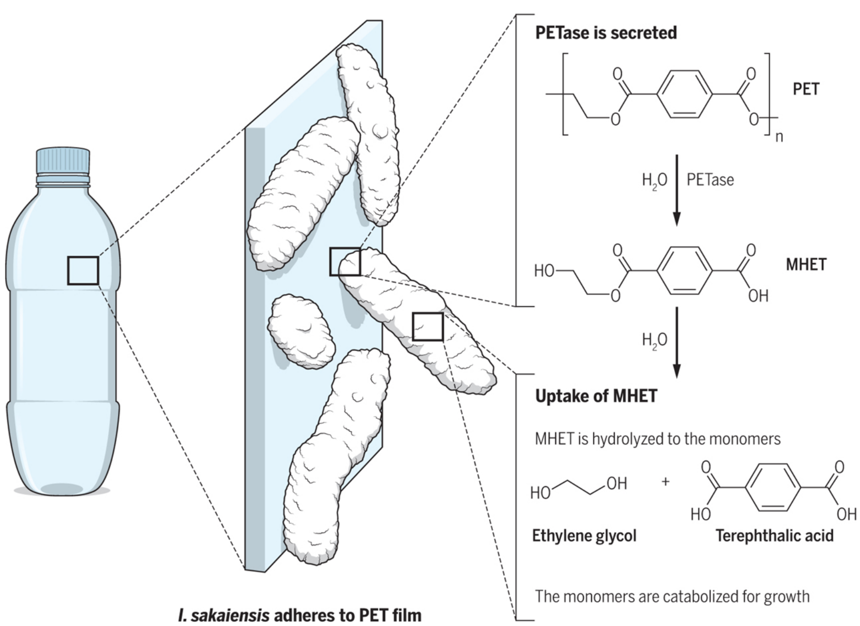

Figure 1: Breakdown of PET by PETase and MHETase. *PETase breaks PET down into MHET, which then enters the cell, where MHETase breaks it down further into ethylene glycol and terephthalic acid. These monomers are funneled into metabolic pathways, allowing* I. sakaiensis *to extract energy and carbon for growth.*

To break PET down into its component monomers, *I. sakaiensis* enlists the help of enzymes, biological catalysts that facilitate chemical reactions in cells. *I. sakaiensis* has two enzymes, PETase and MHETase, which work together to break the bonds connecting PET's monomers to each other. Once broken apart, these monomers can serve as inputs for metabolic pathways, allowing *I. sakaiensis* to extract carbon and energy for growth.[2]

Let this sink in: plastic, the poster child for indestructibility, can now be broken down in the environment.

Paradigm-shifting as this is, it's not too difficult to see how *I. sakaiensis* may have acquired its plastic-degrading ability. We can look to PETase-related enzymes, enzymes that break down natural polymers like cutin, which makes up the waxy coating on plant leaves. Researchers compared the 3D structures of PETase and closely related enzymes to pinpoint specific features of PETase that are responsible for its superior PET-degrading ability.[4] Their analysis suggests that PETase evolved from these closely related enzymes under the selective pressure created by the presence of PET in the environment. Given that PET only entered into widespread use about fifty years ago, this evolution was rapid.[3]

Learning about the evolution of PETase raises a question: how can we build on the work evolution has already done and further improve the enzyme? Presently, PETase is prohibitively inefficient—it takes six weeks for *I. sakaiensis* to fully degrade a PET film.[2] Optimizing PETase would enable its use in biocatalysis: the use of enzymes to catalyze industrial chemical reactions. Specifically, PETase has potential for application in reduction of environmental microplastic pollution and industrial plastic recycling.[5]

To optimize PETase, researchers employed a technique called rational protein engineering. They compared PETase to an enzyme with a desirable quality, then designed and evaluated PETase mutants that incorporated specific structural features of this enzyme. One group of researchers used this technique to improve the thermal stability of PETase—they created mutants that are functional at higher temperatures and over a longer period of time than natural PETase.[6] Thermal stability is a vital characteristic for biocatalysis because PET degradation is easier at higher temperatures.[5] Another group of researchers designed PETase mutants that are better at degrading crystalline PET, a variety found in common products like plastic water bottles.[3] These are promising steps in the quest for an improved PETase.

This research shows that PETase is amenable to improvements, but how would this look in practice? Rather than using the naked, purified enzyme to break down plastic, it's more practical to use a whole-cell biocatalyst: a yeast cell (or any other lab-ready microorganism) that has been tricked into expressing PETase on its surface. A whole-cell biocatalyst has higher PET degradation activity than purified PETase under all tested conditions and for a longer period of time. Using a whole-cell biocatalyst is also more cost-effective than using a purified enzyme, because it retains catalytic activity through multiple rounds of reuse.[7]

Wastewater treatment plants present a possible area for implementation of PETase whole-cell biocatalysts; because wastewater isn't currently treated to remove plastic, microplastics

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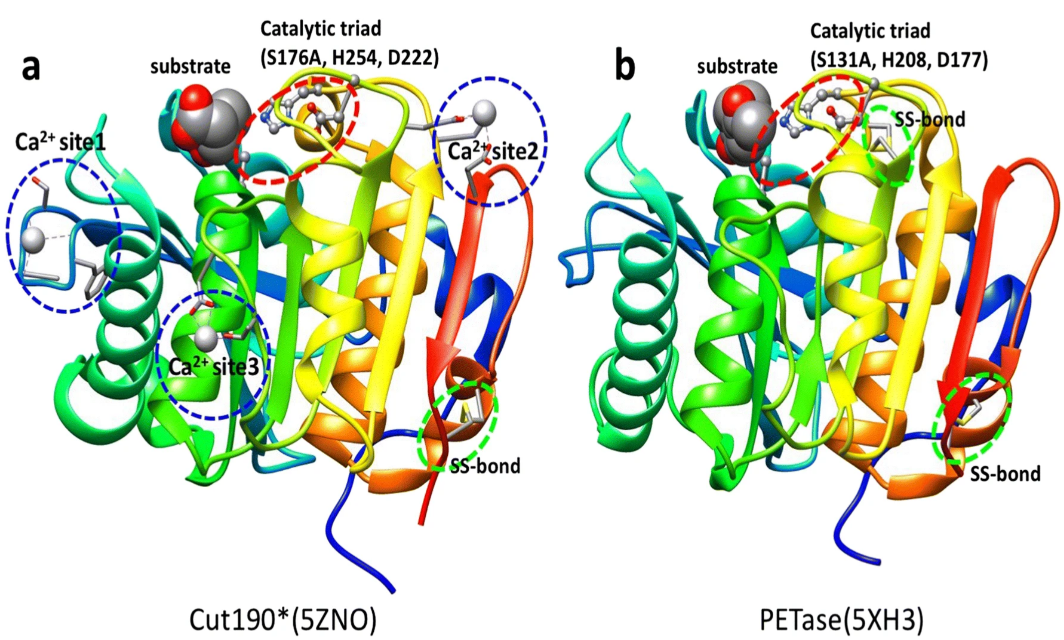

Figure 2: Comparison of the 3D structures of PETase (right) and a related bacterial enzyme, Cut190 (left). *Important components of the enzymes are circled in red, blue, and green. Structural analyses like these allowed researchers to determine the features of PETase responsible for its superior PET-degradation ability. It also provided them with insight on the evolution of PETase. Licensed under CC BY 4.0.*

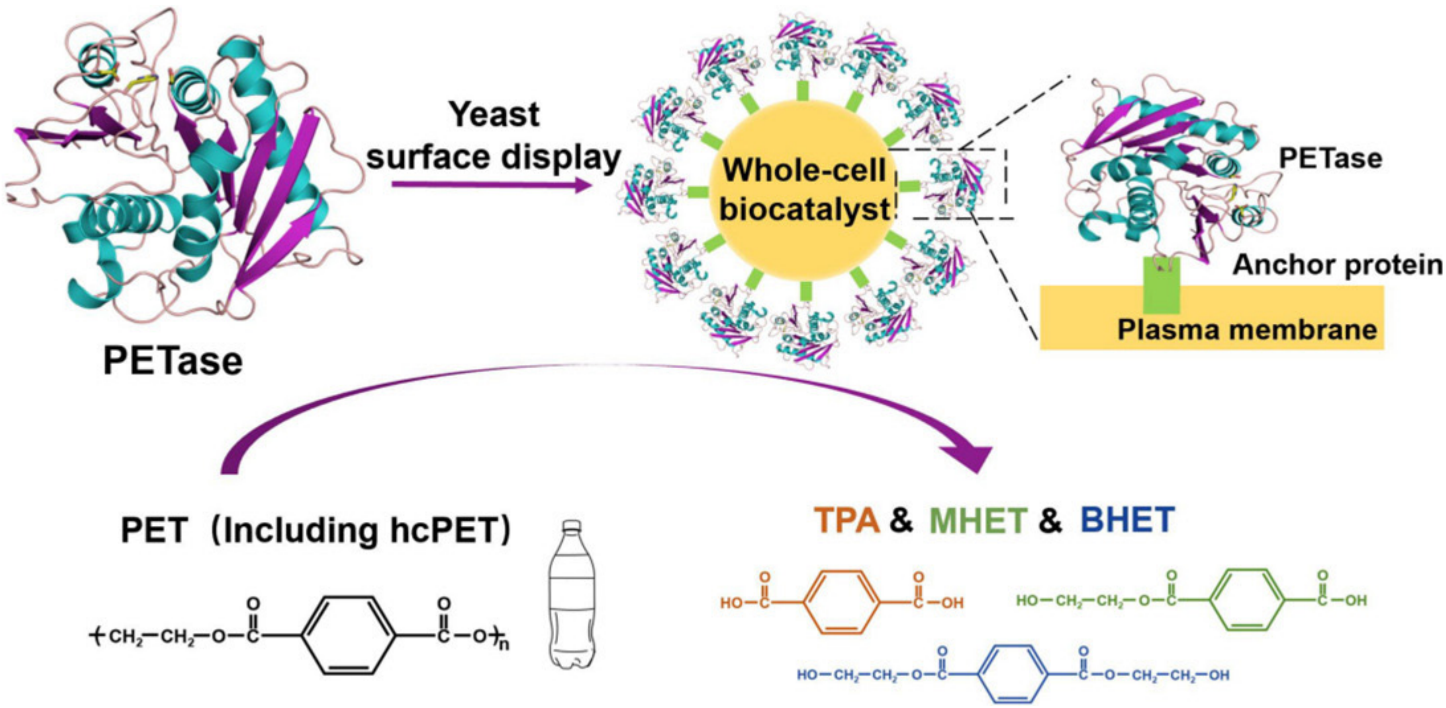

Figure 3: A yeast whole-cell biocatalyst expressing PETase. *PETase must be expressed extracellularly because PET is too large to enter the cell. Whole-cell biocatalysts expressing PETase could be applied to plastic degradation in wastewater treatment plants and industrial plastic recycling.*

from household products and clothes flow all the way into the groundwater and the ocean. The microorganisms that are already used to treat wastewater could be modified to express PETase, greatly simplifying implementation. For whole-cell biocatalysis to be effective, it should be used in conjunction with rational protein engineering to optimize PETase for the specific physical, chemical, and biological conditions of the wastewater treatment system.[8]

Another possible application of biocatalysis is in industrial plastic recycling, which currently faces many challenges.[9] Biocatalysis could be applied to chemical recycling: breaking PET down into its monomers, and then using those monomers to synthesize new polymers.[10] This would contribute to the creation of a circular economy for plastic, greatly reducing use of fossil fuel feedstock and decreasing the amount of plastic waste in landfills and the environment.[11]

This is not a silver bullet solution—we still have a long way to go. PETase only degrades one type of plastic and is still relatively inefficient. Future work should focus on broadening its substrate specificity so that it can be applied to more types of plastic.[6] "Non-hydrolyzable" plastics, such as polyethylene, present an especially daunting challenge because of the tough C—C bonds in their backbones.[12] As bioplastics like polyethylene furanoate gain footing in the plastics economy, it will be important to engineer enzymatic systems that can break them down as well.[3] To discover enzymes that can degrade other types of plastic, we can employ environmental screening methods like the one used to discover *I. sakaiensis*.[8] Finally, before we put these enzymes into use for bioremediation, we must do more research into ecosystem safety and unintended effects.[6]

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Despite these limitations, PETase shows great promise for industrial application, as well as potential for improvement. Tackling the insidious problem of plastic waste will require innovation on many fronts, and implementing biocatalytic degradation can be a valuable contributor to the solution.

Science often looks to nature for inspiration. Sometimes, its inspiration borders on appropriation—we stole penicillin from fungi, and aspirin from willow trees. Why not give *I. sakaiensis* a shot?

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