Engineering enzymes to help solve the planet's plastic problem

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Researchers from the Manchester Institute of Biotechnology (MIB) have developed a new enzyme engineering platform to improve plastic degrading enzymes through directed evolution.

To illustrate the utility of their platform, they have engineered an enzyme that can successfully degrade poly(ethylene) terephthalate (PET), the plastic commonly used in plastic bottles.

In recent years, the enzymatic recycling of plastics has emerged as an attractive and environmentally friendly strategy to help alleviate the problems associated with plastic waste. Although there are a number of existing methods for recycling plastics, enzymes could potentially offer a more cost-effective and energy efficient alternative. In addition, they could be used to selectively breakdown specific components of mixed plastic waste streams that are currently difficult to recycle using existing technologies.

Although promising as a technology, there are considerable hurdles that need to be overcome for enzymatic plastic recycling to be used widely on a commercial scale. One challenge, for instance, is that natural enzymes with the ability to break down plastics typically are less effective and are unstable under the conditions needed for an industrial-scale process.

To address these limitations, in a paper released today in *Nature Catalysis*, researchers from The University of Manchester have reported a new enzyme engineering platform that can quickly improve the properties of plastic degrading enzymes to help make them more suitable for plastic recycling at large scales. Their integrated and automated platform can successfully assess the plastic degradation ability of around 1000 enzyme variants per day.

Dr. Elizabeth Bell, who led the experimental work at the MIB, says that "the accumulation of plastic in the environment is a major global challenge. For this reason, we were keen to use our enzyme evolution capabilities to enhance the properties of plastic degrading enzymes to help alleviate some of these problems. We are hopeful that in the future our scalable platform will allow us to quickly develop new and specific enzymes are suitable for use in large-scale plastic recycling processes."

To test their platform, they went on to develop a new enzyme, HotPETase, through the directed evolution of IsPETase. IsPETase is a recently discovered enzyme produced by the bacterium Ideonella sakaiensis, which can use PET as a carbon and energy source.

While IsPETase has the natural ability to degrade some semi-crystalline forms of PET, the enzyme is unstable at temperatures above 40°C, far below desirable process conditions. This low stability means that reactions must be run at temperatures below the glass transition temperature of PET (~65°C), which leads to low depolymerisation rates.

To address this limitation, the team developed a thermostable enzyme, HotPETase, which is active at 70°C, which is above the glass transition temperature of PET. This enzyme can depolymerise semi-crystalline PET more rapidly than previously reported enzymes and can selectively deconstruct the PET component of a laminated packaging material, highlighting the selectivity that can be achieved by enzymatic recycling.

Professor Anthony Green, Lecturer in Organic Chemistry, says that "the development of HotPETase nicely illustrates the capabilities of our enzyme engineering platform. We are now excited to work with process engineers and polymer scientists to test our enzyme in real world applications. Moving forward, we are hopeful that our platform will prove useful for developing more efficient, stable, and selective enzymes for recycling a wide range of plastic materials."

The development of robust plastic degrading enzymes such as HotPETase, along with the availability of a versatile enzyme engineering platform, make important contributions towards the development of a biotechnological solution to the plastic waste challenge. To move this promising technology forward will now require a collaborative and multidisciplinary effort involving biotechnologists, process engineers and polymer scientists from across the academic and industrial communities. With the world facing an ever-mounting waste problem, biotechnology could provide an environmentally sustainable solution.

More information: Anthony Green, Directed evolution of an efficient and thermostable PET depolymerase, *Nature Catalysis* (2022). DOI: 10.1038/s41929-022-00821-3.
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