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The Answer to Plastic Pollution

How enzymes are the future!

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As we all know, plastics are a crucial material used for decades in many industry sectors such as clothing, health care, electronics and more. It is projected that by 2040, 800 million tons of plastics per year will be produced around the world (Carniel *et al.,* 2021). Many people would be surprised to know that one of the main plastics, polyethylene terephthalate (PET), is found in clothes and when these clothes are washed small plastics known as microplastics are released.

These microplastics are smaller than 5 mm in size and are a troubling concern for the aquatic ecosystem. In a case study in China, PET microplastics were found in a species of mussels (*Mytilus edulis*) along 12,400 miles of coastline (Wu *et al.,* 2017). Microplastics have also been found in human food and beverages, such as seafood, drinking water, salt and sugar, and even in the air you breathe (Henry *et al.,* 2019). This sets the problem of microplastic production into perspective and why solutions to this troubling issue need to be placed at the forefront of biotechnological advances.

The solution to plastic pollution

To eliminate the problem of microplastics such as PET, enzymes able to degrade them can be used to stop microplastics, many of which originate from washing clothes, from being released into the environment. Enzymes are proteins referred to as biological catalysts as their role is to speed up reactions (Robinson, 2015). Indeed, they have active sites where a substrate (the target of the enzyme) binds. You can think of the enzyme's active site as a lock and the substrate as its key.

A bacteria called *Ideonella sakaiensis* was recently found to be functionally involved in the degradation of PET (Yoshida *et al.,* 2016). The bacteria attach to PET and produce an enzyme called PETase which degrades the plastic and breaks it up into smaller units called monomers, such as mono-2-hydroxyethyl terephthalate (MHET). This monomer can then be degraded by another enzyme called MHETase which creates its own monomers, ethylene glycol (EG) and terephthalic acid (TPA) (Yoshida *et al.,* 2016). EG and TPA are not harmful and can be easily recycled, unlike PET (Figure 1).

Where can plastic degrading enzymes be found?

Since PET is a man-made plastic, there is no straight forward naturally occurring enzyme that can degrade it. Therefore, we are forced to discover plastic-degrading enzymes via omics-based strategies. The two main omics-based approaches are metagenomics and proteomics (Zhu *et al.,* 2021).

The metagenomic approach involves recovery of genetic material from an environmental sample and scanning of the thousands of genes present for those that could potentially code for plastic-degrading enzymes. A gene is a small section of DNA on a chromosome that encodes for a particular protein, in this case an enzyme. A gene is like a recipe and the cookbook is the chromosome filled with a variety of recipes. These scans can identify appropriate enzyme candidates based on function or based on the candidate's genetics.

The proteomic approach is where a microbe is grown with and without a plastic substrate. In this way, microorganisms that produce plastic-degrading enzymes can be selected. The proteins produced are isolated and identified. Subsequently, the genes for the proteins identified to be breaking down the plastic can be inserted into bacteria, which then produce the protein in high amounts – this is recombinant enzyme production.

In Figure 2, the two approaches to find plastic-degrading enzymes in the environment are summarised. It is vital that the enzymes discovered are specific to degrading plastics and are not released into the wild because this could cause them to start eating our clothes!

Improving plastic-degrading enzymes

To make sure that the plastic-degrading enzymes are effective enough in breaking down substrate, in our case PET, we can use protein-engineering to enhance the catalytic performance of these enzymes. This means that we alter the enzyme's structure to speed up the breakdown of substrate even more! The goal of improving these plastic-degrading enzymes is their use in the future as the main tool for recycling plastics such as PET.

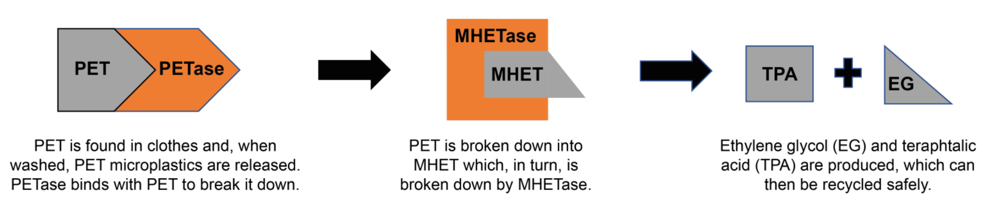

Figure 1. A reaction summarising the breakdown of PET to TPA and EG through the work of enzymes.

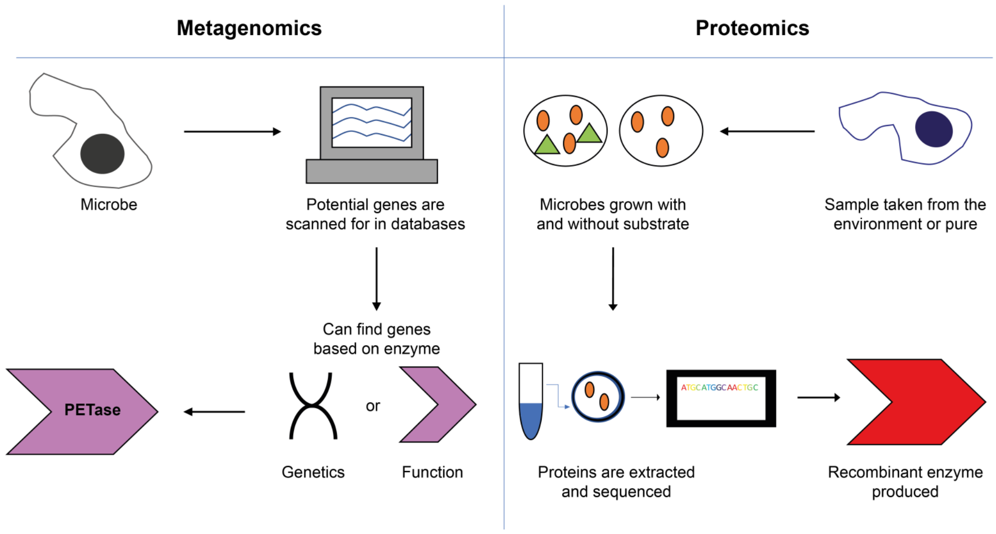

Figure 2. Metagenomic and proteomic approaches to discovering new plastic-degrading enzymes.

There are four main strategies by which the performance of plastic degrading enzymes can be enhanced (Figure 3) (Zhu *et al.,* 2021):

1. Increasing enzyme thermostability

Increasing enzyme thermostability will allow the enzyme to remain intact at higher temperatures. This can be done by forming certain types of bonds in the enzyme structure such as disulphide and hydrogen bonds. These bonds are strong, thereby keeping the enzyme stable at high temperatures such as in your washing machine.

2. Improve substrate binding

Another strategy is improving the efficiency of binding between the enzyme active site and the substrate. This can be achieved by widening the active site to create more room for the substrate to bind; think of it as the bigger the goal the more likely you are to score! To improve efficiency, you can also increase the hydrophobicity of the active site to repel water, which means less water interfering with the active site. A hydrophobic active site is better for the structural integrity of the enzyme.

3. Improving surface interactions

We can enhance these enzymes by improving substrate-enzyme surface interaction. This would mean that the substrate and the enzyme are more drawn towards each other, and it can be achieved by tailoring the electrostatic charge of the enzyme. If something is positively charged, it attracts something negatively charged or repels something of the same charge - this is where the term ‘opposites attract’ comes from. Therefore, if we alter the charge of the enzyme to be neutral, then it cannot repel the substrate away. Increasing surface hydrophobicity also would improve the interaction between enzyme and substrate.

4. Linking enzymes

Finally, the last strategy is to fuse enzymes together to work synergistically. The enzymes would work together simultaneously to break down the targeted substrate, decreasing the time it takes and increasing the efficiency of degradation.

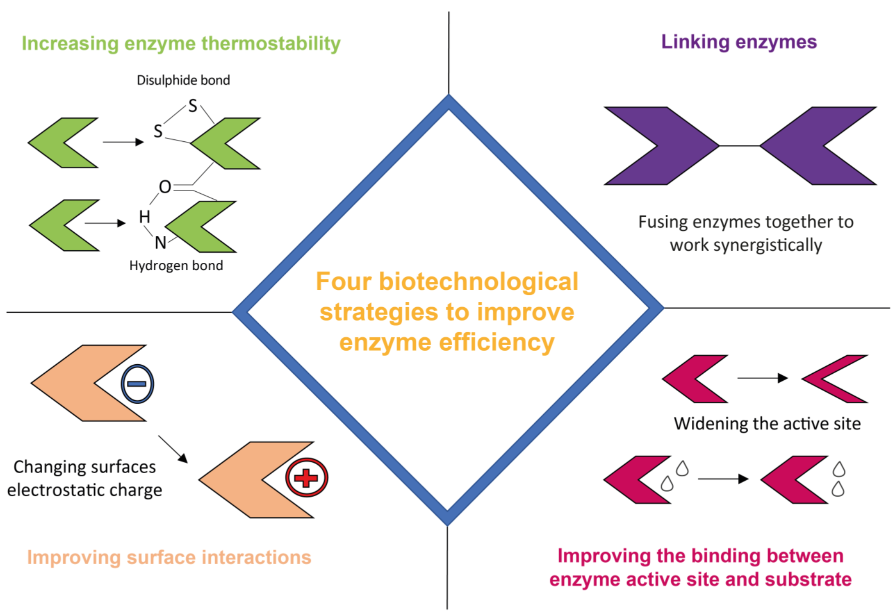

Figure 3. Summary of four biotechnological strategies to improve the efficiency of enzymes.

How close are we?

Using these plastic-degrading enzymes to break up robust big plastics such as PET is a promising step forward in preventing further pollution of this planet. The biodegradation of plastics has been called a "game changer", as substrate breakdown is increased hundred-fold and reaction time is decreased from days to hours. The use of enzyme biocatalysts provides us with a green alternative for managing and recycling our plastic waste sustainably, therefore there would be no need for chemical-dependent recycling. This proves that biodegradation is not only more efficient but more environmentally sound.

However, from an economical point of view, the use of plastic-degrading enzymes as the 'go to' option for recycling plastics such as PET is still a future prospect. Because these enzymes have relatively short lifetimes and are difficult to recover and reuse, it is becoming difficult to introduce them into the mainstream recycling strategies. Therefore, for these enzymes to be used we need to address the problem of regeneration of biocatalysts, and we need to make appropriate preparations to implement this recycling method.

Conclusion

In conclusion, the use of plastic-degrading enzymes is a promising future prospect in managing and recycling robust plastics such as PET. Hopefully, we can solve the economic side of this solution to implement it sooner rather than later - after all, the planet is depending on us!

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