Less Wastage in a Bottle

Jean-Guy Berrin, Marie-Noëlle Rosso, Nicolas Papon

Less wastage in a bottle

Marie Noëlle Rosso [1,*], Jean-Guy Berrin [1], Nicolas Papon [2,3]

[1] INRAE, Aix-Marseille University, Biodiversité et Biotechnologie Fongiques (BBF), UMR1163, Marseille, France.
[2] Host-Pathogen Interaction Study Group (GEIHP, EA 3142), UNIV Angers, UNIV Brest, Angers, France.
[3] Federative Structure of Research « Cellular Interactions and Therapeutic Applications », SFR 4208 ICAT, Univ Angers, Angers, France.

* Correspondance: marie-noelle.rosso@inrae.fr (M.N. Rosso)

Abstract (48 words)

Hundreds million tons of plastics are produced worldwide and accumulate in nature. The thermoplastic resin polyethylene terephthalate (PET) is highly recalcitrant to degradation. A recent report from Tournier and colleagues provides unprecedented advancements towards a circular PET economy by describing an engineered microbial enzyme that efficiently depolymerizes PET.

Keywords: Plastics, white biotechnology, enzyme engineering, circular economy

Core text (973 words)

Circular economy, aimed at avoiding exhaustion of natural resources and the reduction of greenhouse-gas emissions is a major challenge to humankind in the Anthropocene era. In both respects, recycling wastes has key leveraging effect [1]. Petrochemical polymers, including plastics, are essential to the modern society, but are largely non-recyclable. It has been estimated that from the 6,300 Mt of plastic wastes generated until 2017, about 9% have been recycled, 12% have been incinerated, and 79% are accumulating in landfills or marine habitats [2]. For instance, 8 million tons of plastic drift annually into the oceans [1]. As a consequence, industrial countries have engaged in political commitments to reduce plastic wastes. This calls for recyclable and reusable plastics based on green supply chains but also clean manufacture methods and the concurrent development of recycling processes for oil-derived plastics. Land plants produce recalcitrant polymers that minimize water loss, provide structural integrity and protect the tissues from physical or chemical damage and against pathogen attack. During co-evolution with plants, some microorganisms (fungi, bacteria) have developed enzymes able to degrade the highly recalcitrant plant polymers [3-6]. Because plant and synthetic polymers share common chemical and physical properties (e.g. C-C and ester bonds, insolubility in water, high molecular weights), a few microbial enzymes can depolymerize synthetic plastics, albeit with limited efficiencies. This justifies the relevance of exploring microorganisms from diverse environments for the identification of enzymes able to overcome technological burdens.

In a striking new study, Tournier and colleagues used a combination of directed evolution and rational design to engineer enzymes for efficient transformation of colored polyethylene terephthalate (PET), a consumer waste remaining under-valorized due to the lack of a market for this non-standardized colored residue [7]. The implementation of such enzymes in a cost-effective and environmentally-friendly process would greatly improve PET waste management in the frame of circular economy. As a common feature of synthetic polymers, the hydrophobicity, degree of crystallinity, and surface topography are critical issues to enzymatic degradation [8]. In this work, the authors ingeniously got across successive steps of enzyme engineering to overcome these issues (Figure 1A).

They first selected a microbial enzyme (leaf-branch cutinase), based on its performance on amorphous PET and checked for promiscuity activity on bottle-grade PET (Pf-PET). To improve the activity of the enzyme on Pf-PET, they searched for the amino acids involved in substrate binding. Using molecular docking and enzyme contact-surface analysis with 2-hydroxyethyl-monohydroxyethyl terephthalate [2-HE(MHET)] as a model substrate to mimic PET, they identified 15 key amino acids in the hydrophobic groove of the substrate binding site and possibly interacting with the PET substrate. From these, 11 were subjected to site-specific saturation mutagenesis, to generate not less than 209 possible variants. Among these, a couple of variants (referred to as F243I and F243W) showed improved activity on Pf-PET as compared to the wild-type enzyme (Figure 1A).

PET depolymerization requires the enzymes to be active at temperatures approaching the glass transition temperature. The thermostability of the wild-type cutinase (melting temperature 84.7°C in solution) was identified as a limiting factor for Pf-PET degradation. This led the authors to identify the amino acids putatively involved in the binding of divalent ions which could increase the enzyme thermal stability [**9**]. Concerned by the impact of calcium ion addition on the overall cost of enzymatic treatment of tons of substrate, the authors wisely replaced the calcium binding site by a disulfide bond. The enzyme variant referred to as D238C/S283C displayed a melting temperature 9.8°C higher than the wild-type cutinase (94.5°C), albeit with 28% decrease in activity (Figure 1A).

The combination of mutations aiming at disulfide bridge formation and increased specific activity resulted in two new variants with restored activity and upper melting temperatures as compared to the wild-type cutinase. This thermostability was further improved by additional mutations located at the binding site that impacted thermostability after the first round of selection. In fine, four variants were obtained, with specific activities similar to or higher than the wild-type cutinase and melting temperatures improved from +9.3°C to +13.4°C. Molecular-dynamics simulations showed that one of these variants has increased affinity toward 2-HE(MHET) and higher catalytically productive binding with 2-HE(MHET) compared to the wild-type cutinase.

Finally, the authors tested these four enzyme variants at a ton-scale on post-consumer colored PET. Colored PET wastes (PcW-PET) were pre-treated according to technologies widely used in plastic industries (termed extrusion and micronization) to make PcW-PET amorphous and to increase the surface exchange. After adjusting the enzyme concentration to 3 $mg_{enzyme}.g_{PET}^{-1}$, the two best cutinase variants reached 90% conversion in 10.5 h and 9.3 h, respectively, at 72°C, outperforming all PET hydrolases reported hitherto including that of the bacterium *Ideonella sakaiensis* strain 201-F6 [**10**]. Because enzyme cost is a limitation to enzymatic treatments when dealing with tons of substrate, the authors tested the relevance of reducing enzyme concentration to 2 $mg_{enzyme}.g_{PET}^{-1}$ in a 150 liters pilot-scale assay at very high PcW-PET content (200 g kg^{-1} of total reaction weight). In these conditions, the enzyme cost represented only 4% of the ton-price of the virgin PET. The recovered terephthalic monomers were successfully used for recycling, and after discoloration and crystallization, the recycled PET polymers were near colorless, with chemical and mechanical properties similar to that of bottle-grade PET. Importantly, significant quantities of sodium sulfate were retrieved as a co-product of the process, which would improve its economic sustainability.

To summarize, this enlightening article provides an unprecedented global strategy for rationally engineering an enzyme issued from natural biodiversity toward a new biocatalyst with high degradation efficiency on a non-natural polymer. This gives evidence that computer-aided enzyme engineering could represent a powerful strategy to close the loop of the circular economy in a near future (Figure 1B). By considering the industrial constraints throughout the way, the authors reached the objective of proposing a sustainable process for recycling PET wastes that so far threaten Earth habitats.

1. Geng, Y., Sarkis, J., Bleischwitz, R. (2019) How to globalize the circular economy. *Nature*. 565, 153-155
2. Geyer, R., Jambeck, J.R., Lavender Law K. (2017) Production, use, and fate of all plastics ever made. *Science Advances* e1700782
3. Couturier, M. *et al*. (2018) Lytic xylan oxidases from wood-decay fungi unlock biomass degradation. *Nat Chem Biol*. 14, 306-310
4. Paës, G., et al (2019) Tracking of enzymatic biomass deconstruction by fungal secretomes highlights markers of lignocellulose recalcitrance. *Biotechnol Biofuels*. 12, 76
5. Martínez, A.T., *et al*. (2017) Oxidoreductases on their way to industrial biotransformations. *Biotechnol Adv.* 35, 815-831
6. Chen, C., *et al*. (2020) Enzymatic degradation of plant biomass and synthetic polymers. *Nat Rev Chem* 4, 114–126
7. Tournier, V. *et al*. (2020) An engineered PET depolymerase to break down and recycle plastic bottles. *Nature*. 580, 216-219
8. Wei, R., Zimmermann, W. (2017) Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? *Microb Biotechnol*. 10, 1308-1322
9. Then, J. *et al*. (2015) Ca^{2+} and Mg^{2+} binding site engineering increases the degradation of polyethylene terephthalate films by polyester hydrolases from *Thermobifida fusca*. *Biotechnol J.* 10, 592-598
10. Yoshida, S. *et al*. A (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*. 351, 1196-1199

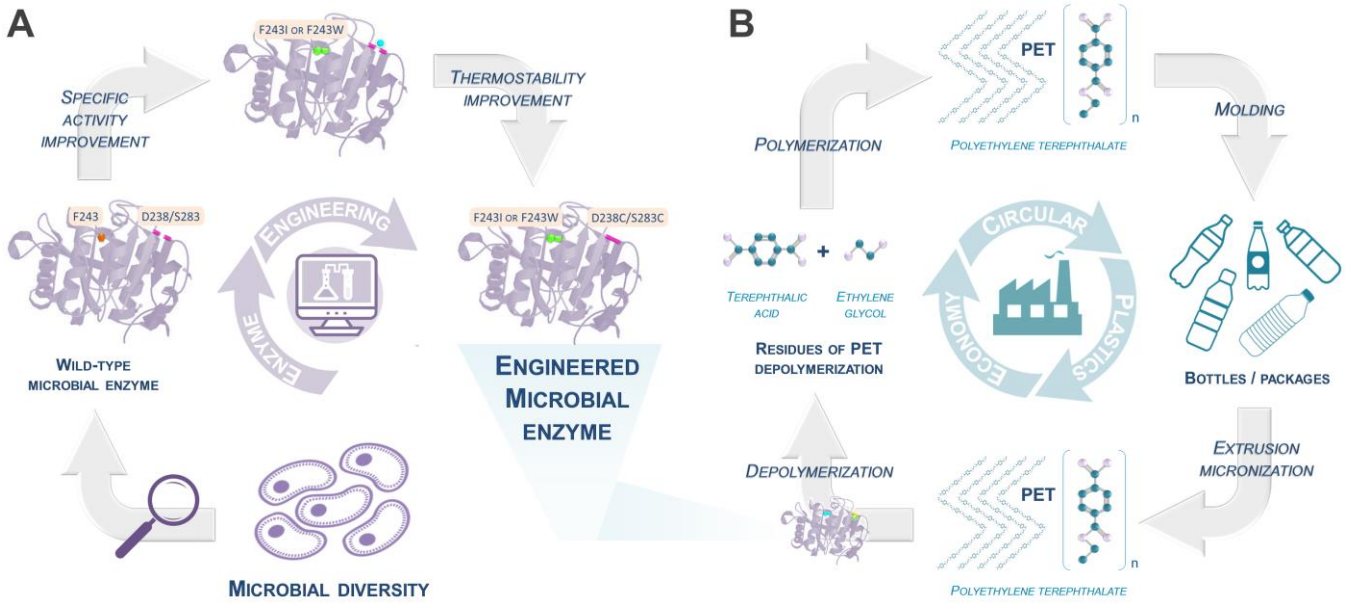

Figure 1. Engineering PET depolymerase to break down and recycle plastic bottles. A. The authors first selected a microbial enzyme, based on its performance on amorphous PET. Using molecular docking and enzyme contact-surface analysis, they identified a couple of variants (referred to as F243I and F243W) showing improved activity as compared to the wild-type enzyme. To increase the enzyme thermal stability, the authors replaced the calcium binding site (D238 and S283) by a disulfide bond (D238C/S283C) leading to an engineered enzyme with improved biochemical properties required for the industrial process. **B.** Computer-aided enzyme engineering represents a powerful strategy to close the loop of the circular economy. By considering the industrial constraints throughout the way, they reached the objective of proposing a sustainable process for recycling PET wastes that so far threaten Earth habitats.