

Rational Protein Engineering of PETase From *Ideonella sakaiensis* to Stimulate the Bioremediation of PET Pollution

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Despite vigorous recycling efforts, nearly 350 million tons of polyethylene terephthalate (PET)—plastic commonly used to package foods and beverages—accumulate annually, posing a serious threat to global ecosystems. To combat this accumulation of plastic pollution, the biological treatment of plastic waste has become an important research focus. This process uses microorganism-derived enzymes to breakdown PET so that new plastic polymers with desirable properties can be sustainably reassembled. Though many hydrolase enzymes can depolymerize plastic, PETase from the bacterium *Ideonella sakaiensis* (IsPETase) is the most promising of these enzymes because of its remarkably high degradation efficiency at moderate temperatures. However, IsPETase's durability and efficiency must be further improved before its ability to serve as a principal recycling strategy can be realized. By utilizing Protein Repair One-Stop Shop (PROSS)—a novel structure-based bioinformatics tool—IsPETase variants with both improved thermal stability and degradation efficiency were developed. Specifically, four unique IsPETase variants were designed and assessed to determine if the PROSS algorithm could effectively improve their stability. In the end, all four variants exhibited higher thermal stability than the wild type, with the most remarkable increase in stability having been demonstrated by variant 3—with a 7.5°C increase in melting temperature. As this is the first study to solely rely on the PROSS algorithm to optimize the stability of IsPETase, this study identifies an exciting new route for developments in protein stability. Further, by gaining this valuable insight into IsPETase's stability, we are closer to developing an effective treatment for pollution.

Awards Won:

Third Award of \$1,000