Improving the Degrading Rate of PETase and Developing Highly Efficient Enzyme Activity Screening Strategies

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Plastic waste poses threats to both the environment and human health. PET is the world's most common plastic. Compared to traditional methods to treat PET waste, biodegradation is environmentally friendly, and its degrading products can be reused. However, the degradation efficiency of current PET degrading enzymes is relatively low for industrial applications. This research aims to develop highly efficient PETase (one of PET degrading enzymes) and its highly efficient enzyme activity screening methods. With degrading substrates and the degrading system standardized, the color fading method was developed to detect the activity of PETase by observing its color change, which, compared to the traditional weighing method, improved the screening efficiency by 60 times. Error-prone PCR was used on the available PETase ICCG to build a mutant library. By using the color fading method to screen enzyme activities first and the weighing method to calculate the exact degrading rate, one mutant was found with its degrading rate 23.9% higher than that of the original ICCG. As the mutant library continued to expand, BHET transparent hydrolysis circle method was developed to screen out the 96% inactive mutants first and focus on the 4% active ones, which further improved the screening efficiency by 25 times. Through sequencing analysis on 75 missense mutations, mutation sites that affect the enzyme activity were summarized and classified, which provided the basis for future rational designs of highly efficient PETase. By verifying that high crystallinity causes incomplete degradation of PET and that high temperature reduces crystallinity, my next research direction was determined to be developing heat-resistant PETases.

Awards Won:
First Award of $5,000