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| Title: | Rational mutagenesis to engineer a cutinase from Thermobifida fusca to improve the degradation of polyethyene terephthalate |
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| Abstract: | Plastic accumulation has become a global issue these days. Polyethylene terephthalate (PET) is the most abundant plastic known. Some microorganisms possess the enzymes that can degrade PET. They use PET as an energy source and convert PET to simpler compounds with no environmental impact. PET hydrolases have been identified in different microorganisms like Tf-Cut2 from Thermibifida fusca, Is-PETase from Idenella sakiensis, and meta-genomically derived like leaf branch compost cutinase (LCC). The work aimed to make the enzyme Tf-Cut2 more catalytically active to degrade PET more efficiently. We used a rational mutagenesis approach to make it more active. The Tf-Cut2 was compared with LCC, and it was found there is a lot of structural and sequence similarity between these two. LCC was one of the best-known PET hydrolases. Through structural superimposition with LCC, Some site-directed mutagenesis was performed in Tf-Cut2. Mainly the mutants made include G62A, G62A/L90F, G62A/F209I, G62A/F249A, and G62A/F249R. The G62A has been included in all mutants as it showed a higher activity when compared to the wild type. Some mutations are found through rational studies that can increase enzymatic activity. Mainly these mutants were made G62A, G62A/L90F, G62A/F209I, G62A/F249A, and G62A/F249R. In all the double mutants, a background of G62A has been taken as it showed a higher activity when compared to wild type 1 . These mutations were done using site-directed mutagenesis via SOE-PCR. The comparison of various mutants was made by looking at the degradation products of PET films at 60°C for 50 hours, and the products are BHET, MHET, and TPA. Apart from this, BHET was also used as a substrate to check the enzyme's catalytic efficiency. A significant increment in the activity has been found in the case of every mutant except F249A. The G62A/F209I mutant showed higher activity among all mutants. Finally, a PET film binding assay is also done to see which mutant can bind with the PET film with higher strength. We have tried to shed light on the mechanism of how a change in a single amino acid can affect its overall structure and catalytic activity through these studies. Using the same mutants, we could identify a couple of crucial amino acids by changing them to other amino acids which lead to a decrease in the activity, and hence they are essential for PET degradation. The mutational studies, done in this work, to make PET degrading enzymes more efficient at their job are beneficial for various industrial and environmental purposes. |
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