Designing Safer Microbial Plastic Degraders: CasTune — An Optimized dCas13d-IF3 Framework for Higher PETase Production in E. coli

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Global PET levels contribute significantly to the world's plastic pollution. A promising biotechnological solution that minimizes harmful byproduct release is utilizing enzymatic degradation of PET through microbial PETase/PET hydrolase. While heterologous hosts like E. coli and P. putida drastically outperform natural Ideonella sakaiensis, significant limitations remain. The aim of this study therefore is to build CasTune, a computational pipeline that increases the PETase production rates in genetically modified E Coli by enhancing the efficiency of pET21b(+)-Is-PETase mRNA with ribosome using catalytically inactive cas13d and the translation initiation factor IF3. dCas13d's usage particularly avoids potential long term problems linked with the previously used cas9 tool, including Horizontal Gene Transfer of transgenic plasmids, Permanent chromosomal changes and double strand breaks as cas13d primarily targets mRNA molecules rather than DNA. In detail, the pipeline involved sequencing the CDS and UTR region of the gene, building a cas13d guide RNA complimentary to the UTR region, binding the cas13d and the IF3 using AlphaFold 2.0 and finally forming a quaternary complex made of dcas13d, IF3, guide RNA and the mRNA using HADDOCK 2.4.The increase in PETase production was investigated by measuring the change in translation initiation rate (measured in initiations per second) after using the complex. Specifically, the dCas13d-IF3 Complex enhanced the translation initiation rate from 0.18 initiations per second to 1.28 initiations per second (assessed through thermodynamic modelling). This proved that CasTune is a scalable, modular framework for optimizing diverse enzymatic pathways beyond the case of PETase and E.Coli.

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