The Deletion of a Disulfide Bridge Through Site-Directed Mutagenesis to Adapt Cutinase to the Biodegradation of Polyethylene Terephthalate

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Plastic pollution is an environmental emergency. Since 1950, over 8.3 billion metric tons of plastic waste has been generated (Asad, 2018). It is estimated that between 75 and 199 million tons of plastic waste is currently in the ocean (Wakefield, 2018). Biodegradation, the use of microorganisms to break down materials, is a promising approach to the plastic problem. One fungus, fusarium solani pisi, secretes a cutinase that has been shown to effectively degrade polyethylene terephthalate (PET), a common plastic (Araujo et al., 2017; Dimarogona et al., 2015; Pirillo et al., 2021; Quartinello et al., 2001; Ronkvist et al., 2009). Cutinase is an enzyme that degrades the naturally-occurring polyester cutin, found in plant cell walls. However, cutinase has low catalytic power, which slows the biodegradation of the plastic (Araujo et al., 2017; Francis Son, 2019). Disulfide bridges, formed between two cysteine residues in an enzyme, increase an enzyme's thermostability, which is its ability to resist denature under high heat (Yin et al., 2015). It has been suggested that the relationship between catalytic power and thermostability is that in order to obtain low activation energy and a more highly-specific active site (which will better fit the substrate and increase catalytic power), it is necessary for the enzyme to become less stable (Roca et al., 2007). This research increased the catalytic power of cutinase through the deletion of a disulfide bridge between Cysteine-31 and Cysteine-109 (C31-C109). The two mutations, C31A (cysteine to alanine) and C31S (cysteine to serine) caused a 55% higher decrease in PET mass compared to the wild-type cutinase over a 24-hour exposure period.

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