ENGINEERING T1 LIPASE FOR DEGRADATION OF POLY-(R)-3-HYDROXYBUTYRATE

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Enzymes with broad substrate specificities that can act on a wide range of substrates would be valuable for industrial applications. T1 lipase is known to have broad substrate specificity in its native form, with active site residues that are similar to polyhydroxylalkanoate (PHA) depolymerase (PhaZ). PhaZ6 from Pseudomonas lemouginii (PhaZ6 Pl) is one of PhaZs that can degrade semicrystalline poly-(R)-3-hydroxybutyrate [P(3HB)]. The objective of this study is to enable T1 lipase to degrade semicrystalline P(3HB) similar to PhaZ6 Pl while maintaining its native function. Structural analyses on PhaZ6 Pl built structure revealed that it does not contain a lid, as opposed to T1 lipase. Therefore, T1 lipase were designed by removing its lid region. This was performed by using Bacillus subtilis lipase A (BSLA) as the reference for T1 lipase modification as the latter does not have a lid region and that its structure fits almost perfectly with T1 lipase based on their superimposed structures. A total of three variants of T1 lipase without lid were successfully designed, namely D1 (without α6–loop–α7), D2 (without α6) and D3 (α6 and loop) in the lid region. All the variants showed PHA depolymerase activity towards P(3HB), with D2 variant exhibiting the highest activity amongst other variants. Further analysis on D2 showed that it was able to maintain its native hydrolytic activity towards olive oil, albeit with decrement in its catalytic efficiency. Results obtained in this study highlighted the fact that native T1 lipase is a versatile hydrolase enzyme which does not only perform triglyceride degradation but also P(3HB) degradation by simply removing the helix 6 which was specifically proven to affect catalytic activity and substrate specificity of the enzyme.