Engineering subtilisin enzymes to depolymerize poly-L-lactic acid plastic films

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Poly-L-lactic acid (PLLA) is a renewably resourced, biodegradable bioplastic that is the most commonly used bioplastic today and will be for the foreseeable future. PLLA is biodegradable in industrial composting conditions, however it is not easily degraded outside these conditions, and composting facilities are not always amenable to taking it. In addition, composting does not circularize the plastic. A better solution would be to return PLLA to the lactic acid monomers from which it is derived. A potential solution for this is to use enzymatic depolymerization to return PLLA to monomers that can then be used to synthesize new PLLA products, creating a true circularization of the product. This recycling application is currently limited by the efficiency of PLLA depolymerizing enzymes. The subtilisin family of serine proteases includes several members that have known abilities to depolymerize PLLA, and our lab discovered PLLA depolymerizing activity in one such protease from the bacterial soil isolate *Bacillus pumilus* B12, and this enzyme was name *Bp*AprE. Expression of *Bp*AprE from a protease-deficient lab strain of *B. subtilis* showed that *Bp*AprE depolymerizes PLLA films significantly better than an orthologous enzyme called *Bs*AprE from a laboratory strain of *B. subtilis* (PY79). *Bs*AprE has substantial protease activity, but no detectable depolymerization activity against PLLA, despite being 76% identical to *Bp*AprE. Using comparisons of the primary sequences and predicted structures of these two enzymes, mutational analyses were conducted to identify key residues that control PLLA depolymerization activity in these enzymes. These studies identified complementary residues in both enzymes that when changed, could improve PLLA depolymerization in both *Bs*AprE and *Bp*AprE. Moreover, combinations of up to 3 simultaneous amino acid changes in the same enzyme led to synergistic increases in the PLLA depolymerizing activities of *Bs*AprE and *Bp*AprE by 830-fold and 230-fold, respectively. Some changes appeared to affect enzymatic activity, but others seemed to influence that ability of the enzymes to bind their substrates. Further work on the *Bs*AprE enzyme revealed that additional amino acid changes that increased binding to PLLA could cause even greater synergistic increases in PLLA degradation. The final combination of 8 amino acid changes in *Bs*AprE caused an ~30,000x increase in activity over the original parent enzyme. Thus, comparative mutational studies on orthologous enzymes with differing abilities to degrade PLLA has proven to be a powerful approach to engineering more effective depolymerizing enzymes with potential applications that will contribute to circularizing the PLLA economy.