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Repertoire of thermostable chimeras engineered by SCHEMA-RASPP for the degradation of bio-based plastics.

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PURPOSE OF THE ABSTRACT

Cutinases (EC 3.1.1.74) are serine esterase enzymes belonging to the superfamily of α/β hydrolases that catalyze the hydrolysis of the ester bond. These enzymes are very attractive for their implementation in industrial processes such as cotton scouring and plastic degradation [1,2]. The increasing interest in cutinases has dramatically enhanced the portfolio of available enzymes (from bacterial and fungus sources) with a variety of features ranging from high thermostability to the ability to hydrolyze different plastic derivatives [3]. From this perspective, it would be worthwhile to combine the properties of the individual enzymes to perform a more efficient degradation of plastics. Enzyme chimeragenesis is a powerful engineering tool aimed at obtaining robust enzymes with enhanced thermostability and broader substrate scope [4]. Among the most successful strategies to generate chimeric proteins, the SCHEMA-RASPP algorithm, developed by the Frances Arnold group [5], has been applied to many enzymes systems, including P450s, β-lactamases, cellulases, arginases, channel rhodopsins and laccases. SCHEMA yields chimeric proteins by homologous recombination of different parental types and complemented by RASPP generates libraries by minimizing 3D disruption of the enzyme for a range of mutations [6].
Here, we present a repertoire of chimeras employing as parental types three cutinases orthologs with 55% sequence identity and diverse biochemical features. Our SCHEMA-RASPP library comprised 7 SCHEMA blocks and 2187 possible combinations. From the family of functional chimeras we preliminary identified 14 designs with striking improvements in thermostability and substrate promiscuity, opening up a venue for future protein engineering enterprises.

FIGURES

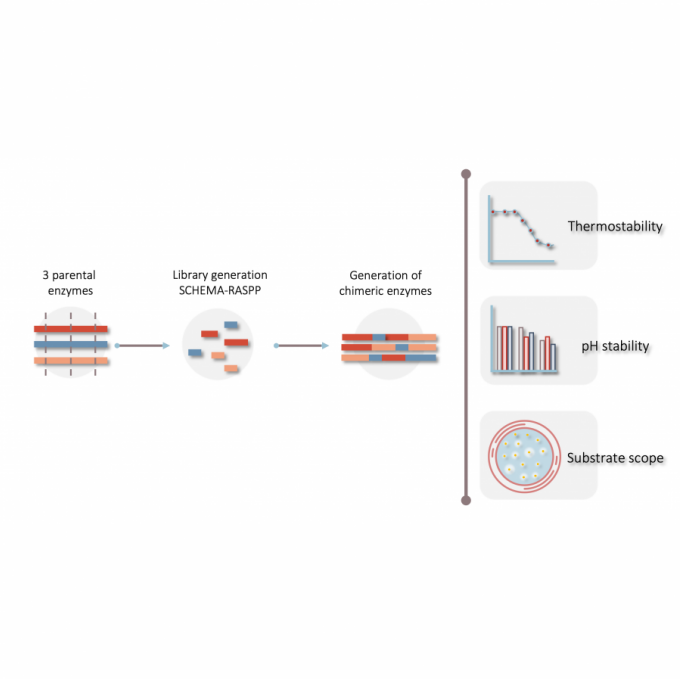

FIGURE 1

Graphical abstract

Graphical abstract of chimeric enzymes generation and their further characterization.

FIGURE 2

KEYWORDS

Chimeragenesis | SHEMA-RASPP | Plastic degradation | Thermostability

BIBLIOGRAPHY

[1] gururaj, p., khushbu, s., monisha, b., selvakumar, n., chakravarthy, m., gautam, p., & d. nandhini, g. prep. biochem. biotechnol. 2020, 51(6), 550-561.

[2] c. knott, b., erickson, e., d. allen, m., e. gado, j., graham, r., l. kearns, f., pardo, i., topuzlu, e., j. anderson, j., p. austin, h., dominick, g., w. johnson, c., a. rorrer, n., j. szostkiewicz, c., copi□, v., m. payne, c., l. woodcock, h., s. donohoe, b., t. beckham, g., & e. mcgeehan, j. proc. natl. acad. sci. u. s. a. 2020, 117(41), 25476-25485.

[3] martinez, a., & maicas, s. 2021. catalysts, 11(10).

[4] heinzelman, p., d. snow, c., wu, i., nguyen, c., villalobos, a., govindarajan, s., minshull, j., & h. arnold, f. proc. natl. acad. sci. u. s. a. 2009, 106(14), 5610-5615.

[5] a. smith, m., & h. arnold, f. methods mol. biol. 2014, 1179, 335-343.

[6] mateljak, i., rice, a., yang, k., tron, t., & alcalde, m. acs synth. biol. 2019, 8(4), 833-843.