Enhancing RGI lyase thermostability by targeted single point mutations - DTU Orbit
(16/12/2018)

Enhancing RGI lyase thermostability by targeted single point mutations

Rhamnogalacturonan I lyase (RGI lyase) (EC 4.2.2.7) catalyzes the cleavage of rhamnogalacturonan I in pectins by β-elimination. In this study the thermal stability of a RGI lyase (PL 11) originating from Bacillus licheniformis DSM 13/ATCC14580 was increased by a targeted protein engineering approach involving single amino acid substitution. Nine individual amino acids were selected as targets for site-saturated mutagenesis by the use of a predictive consensus approach in combination with prediction of protein mutant stability changes and B-factor iteration testing. After extensive experimental verification of the thermal stability of the designed mutants versus the original wild-type RGI lyase, several promising single point mutations were obtained, particularly in position Glu434 on the surface of the enzyme protein. The best mutant, Glu434Leu, produced a half-life of 31 min at 60 °C, corresponding to a 1.6-fold improvement of the thermal stability compared to the original RGI lyase. Gly55Val was the second best mutation with a thermostability half-life increase of 27 min at 60 °C, and the best mutations following were Glu434Trp, Glu434Phe, and Glu434Tyr, respectively. The data verify the applicability of a combinatorial predictive approach for designing a small site saturation library for improving enzyme thermostability. In addition, new thermostable RGI lyases suitable for enzymatic upgrading of pectinaceous plant biomass materials at elevated temperatures were produced.

General information
State: Published
Organisations: Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Department of Systems Biology, Department of Biotechnology, Institute for Product Development, Technical University of Denmark, Chr. Hansen AS
Pages: 9727-9735
Publication date: 2013
Peer-reviewed: Yes

Publication information
Journal: Applied Microbiology and Biotechnology
Volume: 97
Issue number: 22
ISSN (Print): 0175-7598
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.64 SJR 1.182 SNIP 1.161
Web of Science (2017): Impact factor 3.34
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.57 SJR 1.2 SNIP 1.182
Web of Science (2016): Impact factor 3.42
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.43 SJR 1.256 SNIP 1.221
Web of Science (2015): Impact factor 3.376
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.71 SJR 1.332 SNIP 1.448
Web of Science (2014): Impact factor 3.337
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 4.3 SJR 1.54 SNIP 1.43
Web of Science (2013): Impact factor 3.811
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 4 SJR 1.488 SNIP 1.29
Web of Science (2012): Impact factor 3.689