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Switching regioselectivity in the fructooligosaccharides synthesis by Gluconacetobacter diazotrophicus levansucrase

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PURPOSE OF THE ABSTRACT

Fructooligosaccharides (FOSs) are soluble prebiotic fibbers with proven health-promoting effects in humans and animals. Bacterial levansucrases (EC 2.4.1.10) catalyze fructosyl transfer reactions from the natural substrate sucrose to different acceptors, such as water (sucrose hydrolysis), sucrose (FOSs synthesis), and FOSs (polysaccharide formation and elongation). Gluconacetobacter diazotrophicus levansucrase (Gd_LsdA) is distinguishable for the synthesis and accumulation of the β-(2➙1)-linked FOSs 1-kestotriose (1K) and 1,1-kestotetraose (1,1K). The yield of the β-(2➙6)-linked polysaccharide levan is rather low due to the minor proportion of the preferred precursor 6-kestotriose (6K). In this work, the amino acids His172 and Asn306 at the active-site cavity of Gd_LsdA were selected as independent targets for saturation mutagenesis aiming to decipher the structural factors involved in the regioselectivity of the fructosyl transfer reaction. His172 binds the fructosyl moiety of the donor sucrose (subsite -1), while Asn306 interacts with the glucosyl moiety of the acceptor sucrose or the second fructosyl moiety of the acceptor FOS (subsite +2). HPAEC-PAD analysis of the time-course transformation of sucrose by the native Gd_LsdA and the mutated variants revealed remarkable differences in the product profiles. As a general behavior, the replacement of His172 failed to shift the FOSs spectrum and caused a drastic reduction in the ratio of the transfructosylation/hydrolysis activities. More interestingly, the substitution of Asn306 by a positively charged amino acid (Arg or Lys but not His) directed the acceptor sucrose molecule in an orientation that favors the synthesis of 6-kestotriose over 1-kestotriose. Contrary to the native enzyme, the N306R and N306K variants mostly yielded β-(2➙6)-linked FOSs with degree of polymerization (DP) between 3 and 6 which were no further elongated in prolonged reactions. Our results provide insight into a key structural determinant of the regioselectivity and processivity of the fructosyl transfer reaction in levansucrases. On the other hand, the fully active variant N306R yielding the highest 6K/1K ratio constitutes an attractive catalyst for the production of prebiotic levan-type FOSs, which are currently unavailable in the commercial market.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Levansucrase | Fructooligosaccharides | Fructosyltransferase | Fructan

BIBLIOGRAPHY