

EFFECT OF ADDITIONAL DOMAINS ON THE ELONGATION MECHANISM AND FRUCTOSYL LINKAGE SPECIFICITY OF THE MULTIDOMAIN LEVANSUCRASE LevS

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Levansucrase LevS from *Leuconostoc mesenteroides* B-512F is a multidomain fructansucrase, able to produce a highly-branched fructose polymer linked by β 2-6 bonds. Structural architecture of LevS shows three main domains: the N-terminal domain, a region of 155 aa not showing similarity with other proteins; the C-terminal domain shows identity to glycosyltransferases and contains three-fold repeat sequences and a “transition region” located between catalytic and C-terminal domain; lastly, the catalytic domain that contains the active site and is similar to other one-domain fructansucrases (Fig. 1a) [1]. In this study, we explore the structure/function relationship of these additional domains of LevS. Shorter versions of the enzyme were created by deleting both N- and C-terminal domain, as well as the transition region. Our results suggest that these domains are not essential for catalysis, but participate in modulating the transfructosylation activity. Particularly, deleting the transition region has an effect on the transfructosylation activity, reducing transfer-derived products by 20%. Two mechanisms have been described for polymer elongation: the processive mechanism, characterized by the synthesis of high molecular weight (MW) polymer and low production of fructooligosaccharides (FOS); and the non-processive mechanism, producing FOS within a broad MW range increasingly large until reaching the high MW polymer [2]. Product profile analyses show that LevS synthesizes levan by a processive mechanism (Fig. 1b-c). The deletion of C-terminal domain, including the transition region, results in a more hydrolytic enzyme. However, the deletion of the N-terminal domain results in an enzyme catalyzing the synthesis of a low molecular weight (LMW) polymer through a non-processive mechanism. Furthermore, this LMW polymer was identified as inulin, a fructose polymer linked by β 2-1 bonds. Finally, the enzyme containing only the catalytic domain of LevS (devoid of N- and C-terminal domains) synthesizes a LMW polymer of inulin by a non-processive mechanism. These results suggest that the N-terminal domain may be involved in the recognition of the acceptor affecting the linkage specificity.

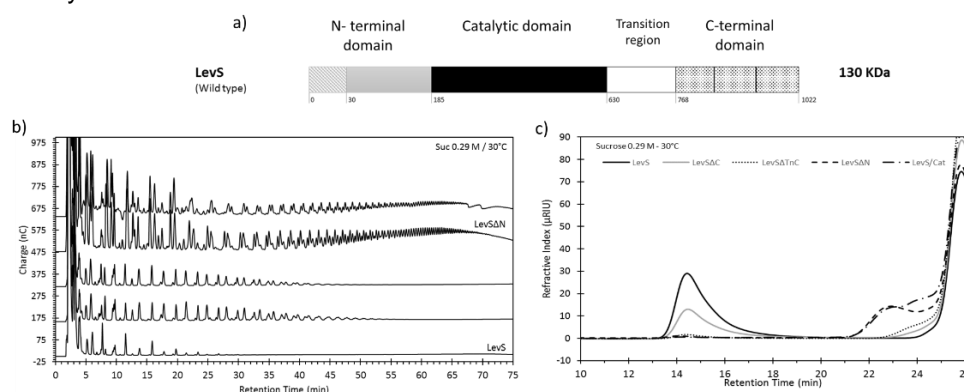


Figure 1. Product profile of LevS and its truncated versions. a) Schematic representation of LevS, b) FOS and c) polymer profile obtained from sucrose by LevS and its truncated versions.

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