

## Enhancing transglycosylation activity and thermostability of levansucrase by proline substitution on flexible coil near Ca<sup>2+</sup> binding site

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Abstract: Levansucrase is a fructosyltransferase that synthesizes levan-type  $\beta$ -2,6-linked fructan (levan) and fructooligosaccharides (LFOS) from sucrose. Structural elucidation of levansucrases showed that this enzyme required Ca<sup>2+</sup> as a cofactor, which some calcium-binding residue located on the flexible coil that links to enzyme active site. In this study, amino acid residues on the flexible coil near the calcium-binding site of *Bacillus licheniformis* RN-01, including G249, D250, N251, and H252, were substituted by proline to increase their stability. The result showed that substitution of G249 by proline significantly increased the melting temperature (T<sub>m</sub>) of the enzyme, while the kinetic stability of both G249P and H252P was increased. G249P could also synthesize a higher MW of levan than that of wild type, while H252P synthesizes very short LFOS with degree of polymerization up to 6. These results indicated that this flexible coil is critical for the stability and transgly-cosylation of levansucrase.

## **Graphical abstract:**



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