Improvement in thermostability and low-temperature catalytic efficiency of *Bispora* sp. β-glucanase through surface charge optimization

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Abstract: Glucanase is one of the most widely used biocatalysts in kitchen waste disposal, biofuel, food and animal feed industries. However, enzyme catalytic activity, thermal stability, and the pretreatment process of biomass are the key factors that affect the efficiency of biomass degradation. Optimization of charge-charge interactions is a structure-based rational design approach that has proven successful in thermostability improvement.

In this study, a structure-based rational design strategy to improve the low-temperature catalytic performance of a highly active GH16 glucanase (BisGlu16B) from *Bispora sp.*MEY-1. Through screening, three locations, E36, D127, and D156, were identified as the contributors to low-temperature catalytic performance and thermostability. All mutants showed improved thermal properties with wild type. Molecular dynamics simulations showed that the mutation of surface charged amino acid aspartate to alanine increased the overall rigidity of protein molecules, reduces the energy of the protein molecule, and increased the number of hydrogen bonds within protein molecules. In addition, the molecular dynamics simulation results are consistent with the experimental.

Keywords: GH16 glucanase, Low-temperature catalytic performance, Thermostability.