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

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## Introduction

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. Generally speaking, glucanase has a high catalytic activity, but its thermal stability cannot yet fully meet the industrial standards. Therefore, it is very necessary to improve the thermal stability of the enzyme on the premise of ensuring its catalytic activity.

## Methods

1, Use over-lap PCR to amplify the high catalytic efficiency glucanase mutant sequence fragment;

2, Clone the glucanase mutant sequence fragment into the EcoR I and Not I restriction sites of the expression vector pPIC9r Between the points, get the recombinant vector;

3, Transform the mutant recombinant vector into Pichia pastoris GS115, induce expression, and obtain the mutant strain;

4, Cultivate the recombinant strain to induce recombinant glucanase expression;

5, Recover and purify the expressed High specific activity glucanase mutant;

Based on the sequence of glucanase wild-type BisGlu16B , the amino acid expression after site-specific mutation was performed by molecular biology technology.

## Results & Discussion

**Table 1 Kinetic parameters and specific activity of BisGlu16B and its two mutants at 37 ℃.**

| Substrate | Barley glucan | | | | | Lichenin | | |
|---|---|---|---|---|---|---|---|---|
| Enzyme | $K_m$ (mg/mL) | $k_{cat}$ ($s^{-1}$) | $V_{max}$ (μmol/min · mg) | $k_{cat}/K_m$ (mL/s · mg) | Specific activity (U/mg) | $K_m$ (mg/mL) | $k_{cat}$ ($s^{-1}$) | $V_{max}$ (μmol/min · mg) |
| BisGlu16B | 4.6 ± 0.13 | 7600 ± 23 | 9700 ± 36 | 1700 ± 17 | 9300 ± 33 | 5.3 ± 0.04 | 5200 ± 22 | 6600 ± 38 |
| E36R | 5.0 ± 0.03 | 9100 ± 7 | 11500 ± 11 | 1800 ± 21 | 10900 ± 89 | 4.3 ± 0.13 | 5600 ± 48 | 7100 ± 74 |
| D127K | 4.9 ± 0.12 | 9900 ± 12 | 12600 ± 20 | 2000 ± 59 | 12100 ± 67 | 4.9 ± 0.04 | 4200 ± 22 | 5400 ± 38 |
| D156A | 3.9 ± 0.03 | 9500 ± 7 | 12000 ± 11 | 2400 ± 21 | 11600 ± 89 | 4.7 ± 0.04 | 5700 ± 22 | 7300 ± 38 |
| E36R/D127K/D156A | 4.4 ± 0.06 | 11700 ± 12 | 14800 ± 18 | 2700 ± 78 | 14300 ± 91 | 5.3 ± 0.02 | 6300 ± 66 | 8100 ± 83 |

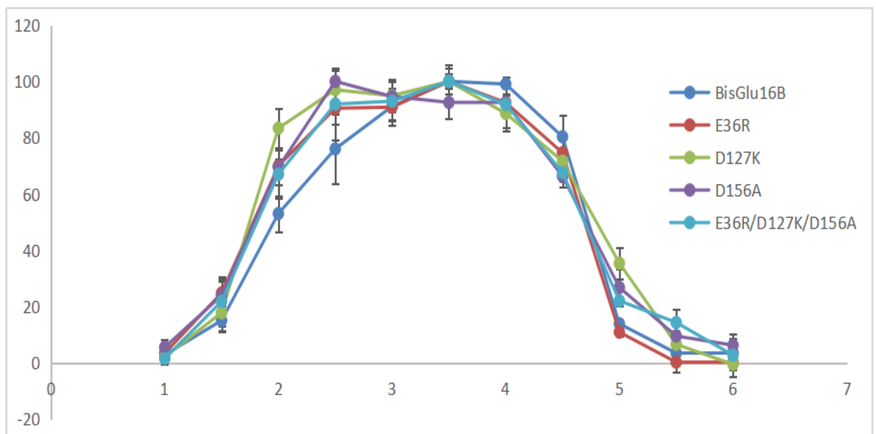


**Fig 1. Optimal pH of the purified recombinant wild-type BisGlu16B and its three mutants**

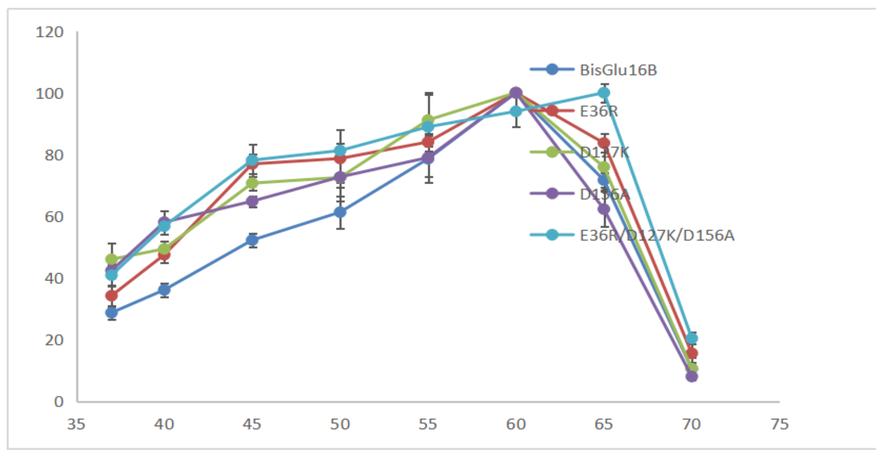


**Fig 2. Optimal temperature of the purified recombinant wild-type BisGlu16B and its three mutants**

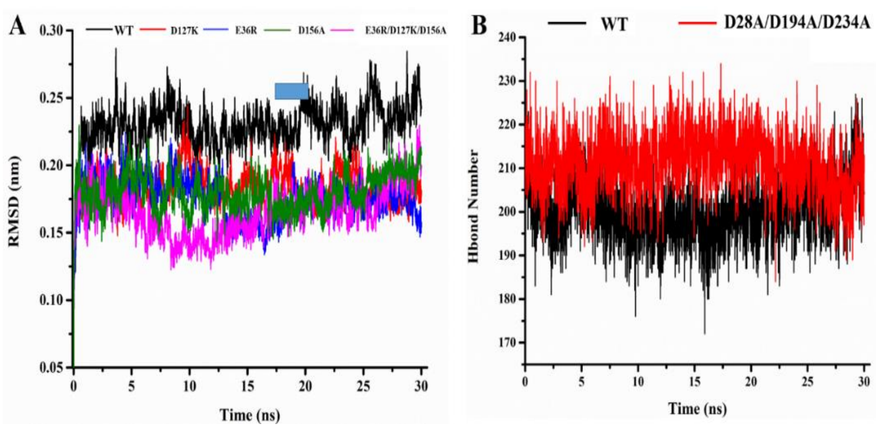


**Fig 3. Molecular dynamics simulation analysis of thermal stability. A. Root mean square deviation (RMSD) of the WT and its mutants. B. The total number of hydrogen bonds within the enzyme molecule changes.**

All mutants showed improved thermal properties, in the order E36R/D127K/D156A > D156A > E36R > D127K > BisGlu16B. 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.

## Conclusion

The experimentally obtained mutant glucanase has higher enzyme catalytic activity under acidic pH and industrial production conditions, and its thermal stability is significantly improved. The charged amino acids on the surface increase the number of hydrogen bonds in the protein molecule and the interaction between the substrate and the protein.

## Acknowledgement

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