

Improvement of thermal stability of β -1,3-1,4-glucanase from *Bispora* sp. MEY-1 by directed evolution

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Introduction

β -D-glucans consist of a heterogeneous group of glucose polymers, comprising β -D-glycosyl residues linked via β -glycosidic bonds, present as structural elements in the cell walls of yeast, fungi and cereal. According to the type of glycosidic bond in a β -glucan, It can be divided into β -1,4-glucan (cellulose), β -1,3-1,4-glucan (lichen chitosan), β -1,3(4)-glucan, and β -1,3-glucan (seaweed polysaccharide). β -1,3-1,4-Glucanase produced by bacteria, fungi, plants and animals (or lichenanase; EC 3.2.1.73) is one of the major enzymes used in industry in recent decades.

Methods

Currently, a variety of protein engineering strategies have been adopted. The T_m value of xylanase was increased by 25°C by directional evolution. The half-life of glucanase was extended three times by surface charge optimization strategy. B-factor value optimization, increasing hydrophobic forces and introducing disulfide bonds are also common methods to improve the thermal stability of enzymes. However, the above methods improve the thermal stability of the enzyme by enhancing the rigidity of the enzyme structure, while losing the catalytic activity of the enzyme [3]. Therefore, how to obtain glucanase with improved thermal stability and catalytic efficiency is very important for the industrial application of enzyme. In this study, BisGlu16B_ΔC, derived from *Bispora* sp. MEY-1, with catalytic efficiency of 18000 mL·s⁻¹·mg⁻¹, was used as the starting material to improve its thermal stability through error-prone PCR strategy. The obtained dominant mutants with improved thermal stability were tested and compared for their enzymatic properties. This study provided reference for the industrial production of glucanase and its application in feed.

Results & Discussion

The kinetic parameters and specific activity of glucanase were determined by using lichenin as substrate at pH4.0 and 55°C. As shown in Table 1, the specific activity and catalytic efficiency of the mutant Q53L were 65% and 22% higher than those of the wt, respectively. The enzyme activities of the other two mutants A43P and T59I were not significantly different from those of the WT.

The enzymatic properties of WT and mutant were determined by using lichen polysaccharide as substrate. As shown in Fig. 1A, The optimal pH of wild-type WT and the three mutants was 4.0, and the pH range was similar, maintaining more than 40% relative enzyme activity between pH2.5-5.0. In terms of pH stability, the pH stability of the three mutants in alkaline environment (pH7.0-9.0) was significantly better than that of the wild type.

Table 1 Kinetic parameters and specific activity of glucanases against lichenin at 55 °C.

Enzymes	K_m (mg/mL)	V_{max} (μ mol/min·mg)	k_{cat}/K_m (mL/s·mg)	Specific activity(U/mg)
WT	3.14 \pm 0.29	64600 \pm 4400	11900 \pm 260	62000 \pm 1300
T40K	2.63 \pm 0.24	43200 \pm 2100	9500 \pm 410	46000 \pm 2500
Q53L	3.98 \pm 0.73	99600 \pm 7400	14500 \pm 840	102000 \pm 830
S311Y	2.76 \pm 0.09	60300 \pm 1500	12700 \pm 150	60000 \pm 960

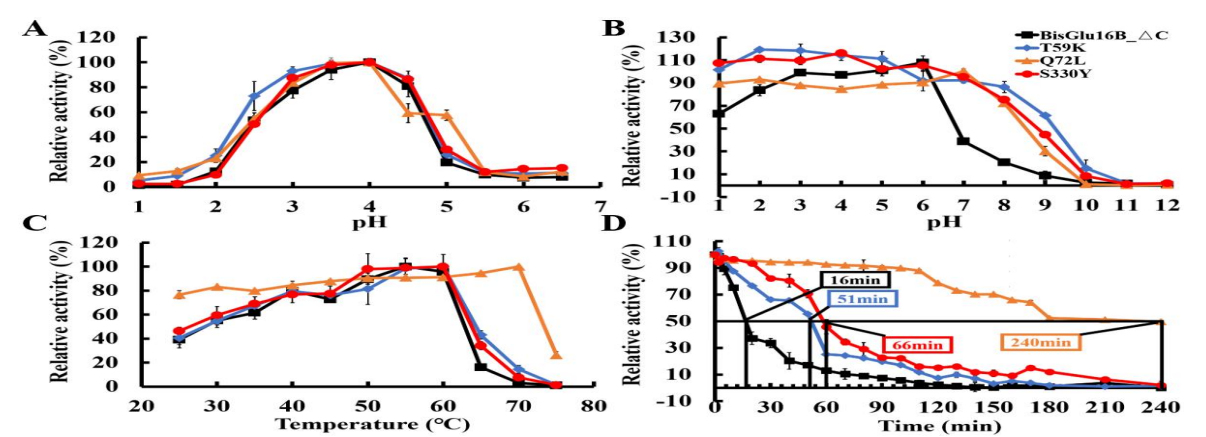


Fig. 1 Properties of the purified recombinant WT and its three mutants. A pH-activity profiles of each enzyme tested at the respective optimal temperatures; B pH-stability profiles; C temperature-activity profiles tested at pH4.0 of each enzyme; D half-lives of the WT and its mutants at 60 °C.

Conclusions

In this study, the thermostability of GH16 β -1,3-1,4-glucanase was improved by directed evolution for the first time. Three mutants with improved thermostability were obtained by high-throughput screening. Enzymatic properties showed that the thermostability and catalytic activity of mutant G165K were improved simultaneously. The dominant mutants obtained in this study show great application potential in the feed industry, and provide a strong reference for the molecular improvement of enzymes.

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