Rational design strategy to optimize the catalytic performance of glucose oxidase to achieve environmentally sustainable development

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Glucose oxidase (GOX) is an oxidoreductase enzyme that utilizes molecular oxygen as an electron acceptor to catalyze the conversion of glucose into gluconic acid (GA) and hydrogen peroxide (H_2O_2) (Tu et al., 2019). It exhibits substrate specificity (Bauer et al. 2022) and offers several advantages such as high catalytic activity and minimal environmental pollution (Bankar et al., 2009). Consequently, it finds wide applications in various industries including food processing, oral hygiene, chemical industry, and medical diagnosis (Zhao et al., 2017). In the textile industry, GOX can be employed in the bleaching process to mitigate environmental pollution and wastewater issues associated with conventional bleaching methods (Jiang et al. 2021). Moreover, in the feed industry, GOX serves as an effective alternative to antibiotics (Dobbenie et al. 1995), helping to prevent environmental problems arising from antibiotic resistance and drug residues (Liang et al., 2023).

To enhance economic benefits, it is essential for the material to maintain stability at higher temperatures and for longer durations. In this study, glucose oxidase GOXA derived from the fungus *Aspergillus eucalypticola* was utilized as the material to ensure the enzyme's catalytic activity, while simultaneously enhancing its thermal stability to meet the requirements of industrial production. Through rational design, we identified 32 potential mutation sites that could impact the thermal stability of GOXA. Subsequently, we screened and identified mutants A12S, A143L, A179V, and L147K, which exhibited improved catalytic performance.

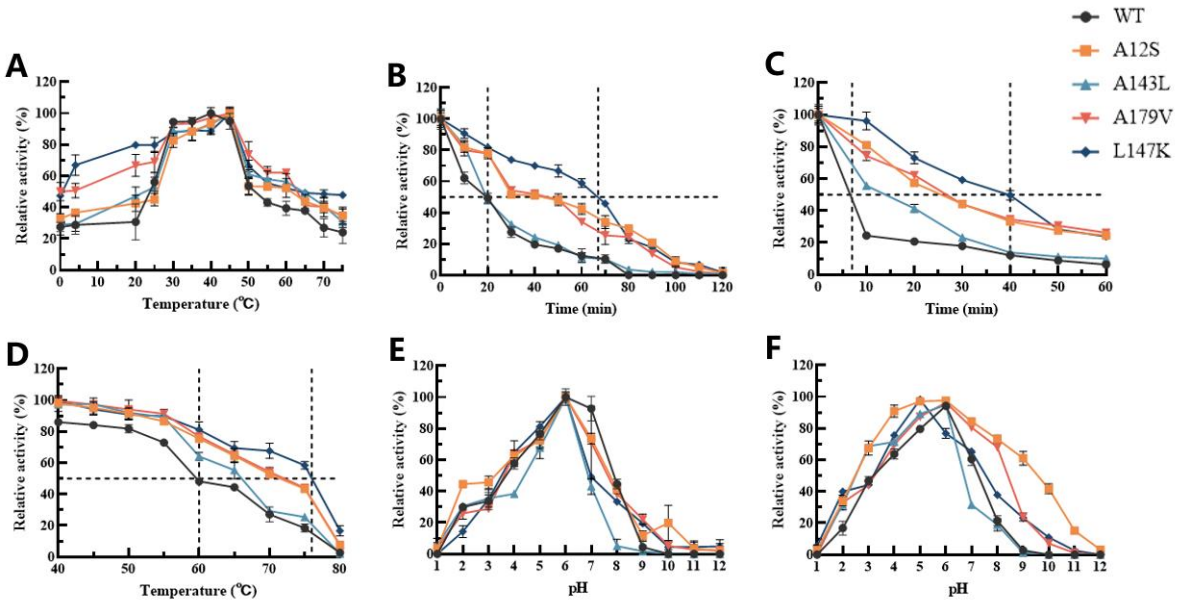

Figure 1 (A) Optimal temperature of wild type and its mutants; (B) Half-life $t_{1/2}$ of wild type and its mutants at 70°C; (C) Wild type GOXA and its mutants at 75°C The half-life $t_{1/2}$; (D) T_{50} of the wild type and its mutants; (E) Optimum pH of the wild type and its mutants; (F) pH stability of the wild type and its mutants.

Fig. 1A shows that the optimal temperature of GOXA is 40°C, and the optimal temperature of the mutants is 45°C. The relative enzyme activity at high temperature (70°C) is significantly higher than that of WT. Fig. 1B

shows that the $t_{1/2}$ of the dominant mutant GOXA_L147K was 68 min at 70°C, which was 2.4 times longer than that of the WT (20 min). Fig. 1C shows that at 75°C, the $t_{1/2}$ of GOXA_L147K is 40 min, which is 4.7 times longer than WT (7 min). As shown in Fig. 1D, the T_{50} value of the wild type is 60°C, and GOXA_L147K is 16°C higher than that of the WT. Mutant GOXA_L147K has the best thermal stability, which may be due to the change of Leu to Lys, making the protein structure more stable. As can be seen from Fig.1E and 1F, in alkaline environments, the pH stability of GOXA_A12S and GOXA_L147K is better than that of the wild type, which is beneficial to their application in the textile bleaching industry. In addition, the specific activities (U/mg) of mutants GOXA_L147K and GOXA_A179V were increased by 5 times and 5.3 times respectively compared with the wild type.

Table 1. Kinetic parametersa of wild-type GOXA and its mutants.

	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}s^{-1}$)	V_{max} (U/mg)
GOXA	24±3.9	388±10.2	16±2.2	34±0.9
GOXA_A12S	18±0.6	584±5.5	33±0.6	84±0.8
GOXA_A143L	38±2.3	777±17.1	21±0.8	139±3.1
GOXA_A179V	19±0.7	1047±2.2	56±1.8	215±0.5
GOXA_A147K	28±0.7	923±0.7	33±0.8	199±0.2

In summary, the mutant L147K exhibits superior catalytic performance compared to the wild type. This study successfully enhanced the catalytic efficiency and stability of glucose oxidase, thereby establishing a theoretical foundation for molecular improvement of glucose oxidase. Furthermore, these findings offer additional options for industrial production and mitigate potential environmental risks associated with the production process.

Acknowledgements

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