Construction and immobilization of thermostable protease mutants for juice clarification

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The annual output of silkworm chrysalis in China exceeds 300,000 tons, accounting for about 80% of the world total(Li et al., 2020). Rich in oil, protein, chitin, sugar, vitamins, purines and rich trace elements, silkworm pupa has high nutritional and health value, studies have shown that silkworm pupa has positive effects on liver protection, immune enhancement, anti-apoptosis, anti-tumor, antibacterial, blood sugar and lipid regulation, and blood pressure reduction(Zhou et al., 2022).

At present, the production of silkworm pupa protein polypeptides is more inclined to enzymatic hydrolysis because of the pollution caused by the traditional chemical degradation process(Ge et al., 2022). The polypeptide products obtained by enzyme catalysis have high antioxidant and other biological activities(He et al., 2022). The researchers also used multiple enzyme synergies to improve the degradation rate and yield(Sarkar et al., 2023). However, the traditional enzymatic hydrolysis system is difficult to separate the product from the catalyst, which results in the waste of enzyme and product to a large extent(Jafari et al., 2020). Meanwhile, there are few researches based on improving the stability of protease itself and immobilizing protease to achieve degradation of silkworm pupa protein and separation of products.

Herein, we designed protease mutants based on high activity serine protease Protease K(PROK)(Ren et al., 2020). Then, casein was used as substrate to determine the differences in pH stability and thermal stability between wild-type protease and mutant protease, which is helpful to obtain better working conditions when using double-enzyme co-catalysis.

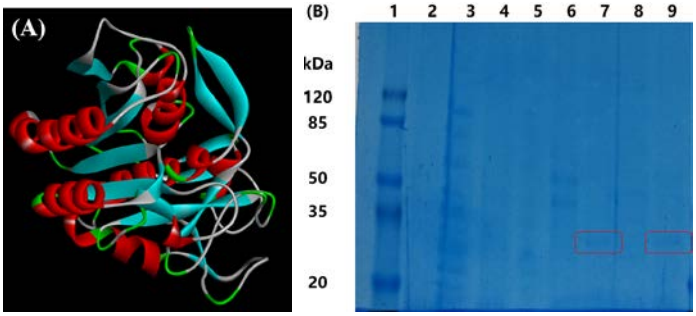

Figure.1 (A) molecular docking simulation of protease mutant structure (B) SDS-PAGE of PROK and PROK mutant; line 1: marker; line 2, 3: crude enzymes; line 4, 5: half-purified enzymes; line 6, 8: waste liquid, line 7, 9: purified enzymes

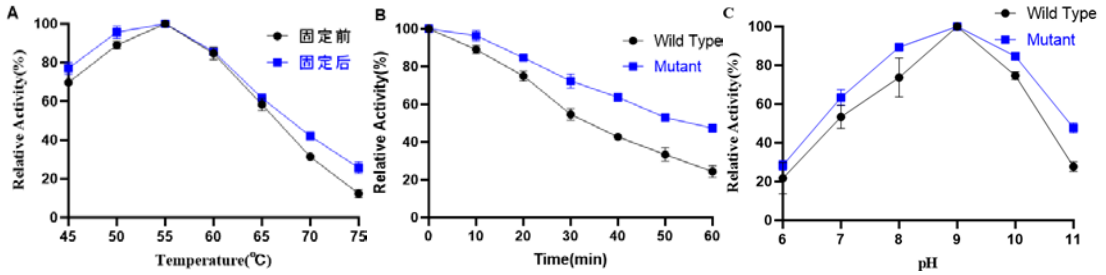

Figure 2 (A) Temperature activity of PROK and PROK mutant; (B) Temperature stability of PROK and PROK mutant at pH 9; (C) pH activity of PROK and PROK mutant at 55℃;In summary, a novel mutant protease with high stability was developed for catalytic degradation of silkworm pupa protein. Next, the influence of co-immobilization of multiple proteases on the degradation effect of silkworm pupa protein will be discussed. It is of great significance to obtain bioactive substances from silkworm pupa protein which is by-product of sericulture industry.

The results of enzyme activity assay showed that the protease mutant improved the enzyme temperature and pH stability compared with the wild type, which was helpful for the degradation of silkworm pupa protein for reuse.

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