DEVELOPMENT OF SCREENING METHOD FOR THE SELECTION OF MUTANTS TO IMPROVE THE SUBSTRATE SPECIFICITY OF *PYROCOCCUS FURIOSUS* THERMOSTABLE AMYLASE

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Key Words: enzyme engineering, saturation mutagenesis, screening, amylase, cyclodextrin

Pyrococcus furiosus thermostable amylase (PFTA) shows the activities of both a cyclodextrin hydrolyzing enzyme and an α-amylase. To improve the substrate affinity and hydrolyzing activity against cyclodextrins, the saturation mutagenesis on the residue of PFTA active site was carried out. After the mutagenesis, the new screening method was needed to select appropriate mutants efficiently from various mutants. Among the α-, β-, γ-cyclodextrins, only β-cyclodextrin makes the complex with phenolphthalein. When added the β-cyclodextrin into phenolphthalein reagent, the color of the solution was changed red to colorless under alkaline condition. In this study, we developed screening method by using 24-well plate and phenolphthalein to compare the activity of PFTA mutants. *Escherichia coli* MC1061 was used as a host for the expression of various recombinant plasmids and cultured in 24-well plate with Luria-Bertani broth containing kanamycin. After cell lysis by heat treatment, each cell extracts were reacted with β-cyclodextrin at 70℃. Reacted mixtures were put into 96-well plate with NaOH solution and then add the phenolphthalein reagent respectively. Lastly, the absorbance of the mixture was measured at 550 nm. The substrate specificity of PFTA mutants was compared from the difference of absorbance.

References

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