Abstract

Phytases catalyze the hydrolysis of the phosphate moieties from phytic acid. . Phytases are used as feed additive for simple-stomached animals, such as swine and poultry. Addition of this phytase to feed substantially improved phosphorus utilization reduced excretion of phosphorus in the feces. . Previous studies showed that site-directed mutagenesis of Q27 (Q50 in *A. niger* TR170 phytase) of *Aspergillus fumigatus* phytase had a significant effect on specific activity, pH activity profile and substrate specificity. Amino acid sequence alignment of *A. fumigatus* phytase and *A. niger* TR170 phytase (PhyA 170) showed that Q50 is strictly conserved. By using the crystal structure of *A. niger* NRRL 3135 phytase at 2.5Å resolution as the template structure, the model structure of *A. niger* TR170 phytase (PhyA170) was built and found Q50 in the area of active site. In this study we used site-directed mutagenesis to analyze the function of Q50 of PhyA170. Q50 was substituted to nine amino acids; Q50A, Q50G, Q50I, Q50L, Q50N, Q50P , Q50T, Q50V, and Q50S. All mutants can be expressed as 66 kDa recombinant proteins in *Pichia pastoris* and the catalytic activity of nine mutant proteins were investigated. We found that Q50A, Q50I, Q50N, Q50P , Q50T, Q50V, and Q50S mutants as well as the wild-type have optimal temperature and pH for phytase activity at 50°c and pH 5.5, respectively. Q50G, and Q50L mutants have relatively the same optimal temperature as other mutants but their optimum pH is 4.5. Q50P mutant has the highest specific activity compared to other mutants and higher than the wild-type phytase at 50°C, pH 5.5. All of nine mutants still contain broad pH stability and thermostability especially Q50P mutant which has higher broad pH stability and thermostabily than that of wild type. Q50P mutant showed higher K_m , V_{max} and k_{cat} than the wild-type suggested that the rate of catalytic reaction of Q50P mutant is higher than that of wild-type but the stability of Q50P mutant structure in complex with phytic acid is less than the wild-type. The model of phytic acid-phytase complex showed that substitution of Q50 by Leucine caused interfere of enzyme-

substrate binding by the longer sidechain of Leucine and substitution of Q50 by Proline which has relatively the same side chain as Glutamine has lower effect in enzyme-substrate binding. Q50P mutant has higher k_{cat} than in wild-type enzyme may caused by the less distance between catalytic residues (R85, D362) of Q50P and oxygen atom of target phosphate than in wild-type. The results suggested that Q50 facilitates the hydrolysis of phosphodiester bond to liberate the phosphate group as a product from the active site of *A. niger* TR170 phytase (PhyA170).