Improving Activity of Honeybee α-Glucosidase III by Substitution of Q349 and L350 Position.

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**Purpose** European honeybees possess 3 α-glucosidase isozymes (HBG I, II and III) belonging to the GH13 family. HBGIII is characterized by the ability to hydrolyse sucrose and maltose with much higher $K_m$, than the others do in spite of high sequence similarity. In this study, Q349 and L350 adjacent to the third catalytic residue D348 were replaced by naturally occurring residues to reveal the involvement of the residues in the activity. **Methods** Single mutants (Q349R/N/Y/I, L350V/H) and double mutants (Q349R/L350V, Q349N/L350H) were produced by *Pichia pastoris* and purified to homogeneity. Kinetic parameters for sucrose, maltose and pNPG, and initial velocities for various substrates were determined. **Results** The single mutant L350H showed the most favorable to all substrates and showed the additive effect when combination with Q349N resulting in increase of $k_{cat}/K_m$ value by 6.44 -fold compare with wild type enzyme to sucrose. This finding suggested that the amino acids substitution at the position of 349 and 350 caused different effects on sucrose binding.