PhaZ7 is an extracellular native poly-R-hydroxyalkanoate (nPHA) depolymerase that acts on the short chain length poly-(R)-hydroxyalkanoates (SCL-PHA). Site-directed mutagenesis studies have been carried out to study the effect of specific amino acids in PHA hydrolysis. In particular, Tyr105, which has been suggested to play a role in PHA adsorption to the enzyme, was mutated to Ala, Glu and Phe. Crystal structures of wild-type PhaZ7 (WT), Y105E and Y105A were determined using X-ray crystallography. The proteins were crystallized in different conditions using the PACT crystallization screening. Data were collected on the X11 and X13 synchrotron beam lines in EMBL Hamburg. Detailed structural comparisons between these structures and previously solved structures of PhaZ7 were carried out.

The crystal structures of Y105E, Y105A and WT were determined at 1.60 Å. In all structures, there are two chains (A and B) of the protein in the asymmetric unit. Structural analysis of WT PhaZ7 and Y105E showed a drastic conformational change in a loop within the region 280-296 of chain A in the structure. Moreover, the residues Trp252 and Phe251 in Y105E were found to have swap places compared to the WT. The residues in the loop 247-253 follow a different path in the two structures. However, no such conformational changes were observed in the Y105A mutant. The results provide a structural explanation of the reduced PHA binding activity of the Y105E by inducing conformational changes that alter the surface of the enzyme.

Keywords: Depolymerase enzymes, PHAs, PhaZ7, Y105E, Y105A, Crystals, X-ray crystallography, conformational change