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ENHANCING THERMAL TOLERANCE OF *ASPERGILLUS NIGER* PHYA PHYTASE DIRECTED BY STRUCTURAL COMPARISON AND COMPUTATIONAL SIMULATION

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Phytase supplied in feeds for monogastric animals is important for improving nutrient uptake and reducing phosphorous pollution. High-thermostability phytases are particularly desirable due to their ability to withstand transient high temperatures during feed pelleting procedures. A comparison of crystal structures of the widely used industrial *Aspergillus niger* PhyA phytase (AnP) with its close homolog, the thermostable *Aspergillus fumigatus* phytase (AfP), suggests eighteen residues in three segments associated with thermostability. In this work, we aim to improve the thermostability of AnP through site-directed mutagenesis. We identified favorable mutations based on structural comparison of homologous phytases and molecular dynamics simulations. A recombinant phytase (AnP-M1) was created by substituting eighteen residues in AnP with their AfP analogs. AnP-M1 exhibited greater thermostability than AnP at 70°C. Molecular dynamics simulations suggested newly formed hydrogen bonding interactions with nine substituted residues give rise to the improved thermostability. Thus, another recombinant phytase (AnP-M2) with just these nine point substitutions was created. AnP-M2 demonstrated superior thermostability among all AnPs at ≥70°C: AnP-M2 maintained 56% of the maximal activity after incubation at 80°C for 1 h; AnP-M2 retained 30-percentage points greater residual activity than that of AnP and AnP-M1 after 1 h incubation at 90°C. The resulting AnP-M2 is an attractive candidate in industrial applications, and the nine substitutions in AnP-M2 are advantageous for phytase thermostability. This work demonstrates that a strategy combining structural comparison of homologous enzymes and computational simulation to focus on important interactions is an effective method for obtaining a thermostable enzyme.

Biography

Zunxi Huang has completed his PhD from Jiangnan University, P R China. He is now the Vice Dean of School of Life Sciences in Yunnan Normal University. He is the Director of Engineering Research Center of Sustainable and Utilization of Biomass Energy approved by Ministry of Education of P R China. He has over 200 publications and more than 40 authorized patents and has been serving as an Editorial Board Member of reputed journals.

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