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Molecular Docking and Site-directed Mutagenesis of a Bacillus thuringiensis Chitinase to Improve Chitinolytic, Synergistic Lepidopteran-larvicidal and Nematicidal Activities

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Abstarc :

Bacterial chitinases are useful in the biocontrol of agriculturally important pests and fungal pathogens. However, the utility of naturally occurring bacterial chitinases is often limited by their low enzyme activity. In this study, we constructed mutants of a Bacillus thuringiensis chitinase with enhanced activity based on homology modeling, molecular docking, and the site-directed mutagenesis of target residues to modify spatial positions, steric hindrances, or hydrophilicity/hydrophobicity. We first identified a gene from B. thuringiensis YBT-9602 that encodes a chitinase (Chi9602) belonging to glycosyl hydrolase family 18 with conserved substrate-binding and substrate-catalytic motifs. We constructed a structural model of a truncated version of Chi9602 (Chi960235-459) containing the substrate-binding domain using the homologous 1ITX protein of Bacillus circulans as the template. We performed molecular docking analysis of Chi960235-459 using di-N-acetyl-D-glucosamine as the ligand. We then selected 10 residues of interest from the docking area for the site-directed mutagenesis experiments and expression in Escherichia coli. Assays of the chitinolytic activity of the purified chitinases revealed that the three mutants exhibited increased chitinolytic activity. The ChiW50A mutant exhibited a greater than 60 % increase in chitinolytic activity, with similar pH, temperature and metal ion requirements, compared to wild-type Chi9602. Furthermore, ChiW50A exhibited pest-controlling activity and antifungal activity. Remarkable synergistic effects of this mutant with B. thuringiensis spore-crystal preparations against Helicoverpa armigera and Caenorhabditis elegans larvae and obvious activity against several plant-pathogenic fungi were observed.

Key Word :
Chitinase; Bacillus thuringiensis; Homology modeling; Molecular docking; Site-directed mutagenesis; Synergistic activity

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