

## **Structural Insights into the Enzymatic Activity of Cysteine Protease Bromelain of MD2 Pineapple**

### **ABSTRACT**

Background and Objective: The MD2 pineapple contains 14 various sizes of bromelain (MD2-bromelains) ranging from 19-200 kDa which are suspected to be structurally and enzymatically varied. This study aims to compare the enzymatic activity and structural features of small and medium-sizes of MD2-bromelains, designated as MD2-SBro (19 kDa) and MD2-MBro (38 kDa), respectively. Materials and Methods: Purified recombinant MD2-SBro and MD2-MBro obtained were used in this study. The enzymatic activity of both MD2-bromelain was determined using a plate agar system with casein as a substrate. Three-dimensional (3D) structures of both MD2-bromelains were constructed under SWISS-MODEL server-based structural homology modeling and verified stereo-chemically. Results: The MD2-SBro and MD2-MBro were shown to be enzymatically active toward casein with MD2-MBro exhibited higher enzymatic activity than MD2-SBro. The 3D structures revealed that Cys-His active site position of MD2-SBro was found to be located in the inappropriate location for catalysis. Besides, the substrate-binding pocket of MD2-SBro was found to be less hydrophobic than that of MD2-MBro. Conclusion: Unique structural features around the active site of MD2-SBro and MD2-MBro might account for the discrepancy in their enzymatic activities.