

*Bacillus pumilus*. Compared with the wild-type DHAP hydrolytic activity, the double-site variant W106K/V149I showed an increase in specific hydrolytic activity at 15 °C by 2.3-fold toward casein in terms of hydrolytic rate and 2.7-fold toward the synthetic peptide AAPF-pN by means of  $k_{cat}/K_m$  value. The thermostability of the variant (W106K/V149I) was improved with the half-life at 60 and 70 °C increased by 2.7- and 5.0-fold, respectively, when compared with the thermostability of the wild-type DHAP. Conclusively, an increase in the cold activity and thermostability of a bacterial alkaline protease was achieved by protein engineering.

This article mainly describes the protease of DHAP can improve thermostability and activity through the site combination.

